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Dithranol-mediated, dose-dependent priming and activation of luminol-enhanced chemiluminescence responses of human neutrophils *in vitro*

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SUMMARY

At concentrations of 10 μg/ml and greater, dithranol (anthralin) caused an intense, dose-related activation of luminol-enhanced chemiluminescence (LECL) of human neutropils and also increased oxygen consumption by these cells. Activation of LECL, which was maximal with 40 μg/ml dithranol, occurred promptly, peaked at 3–5 min, then declined. Dithranol-mediated stimulation of LECL was inhibited by catalase and by the protein kinase C inhibitor, H-7. Neutrophils from individuals with chronic granulomatous disease were unresponsive to the pro-oxidative effects of dithranol. At concentrations of 2·5 μg/ml and less, dithranol did not directly activate the LECL responses of neutrophils. However pre-treatment of neutrophils with dithranol at concentrations of 0·5–2·5 μg/ml increased the LECL-responses of cells subsequently stimulated with calcium ionophore and opsonized zymosan. These observations demonstrate two distinct dose-related, pro-oxidative interactions of dithranol with human neutrophils: low-dose priming and high-dose activation of oxidant generation. Since phagocyte-derived reactive oxidants are immunosuppressive, these pro-oxidative interactions of dithranol with human neutrophils may contribute to the pharmacotherapeutic mechanisms of this agent.

The pharmacotherapeutic mechanisms of the widely-used anti-psoriasis agent, dithranol, have not been firmly established. It has been proposed that free radicals, generated by auto-oxidation following topical applications, may mediate both the therapeutic and irritant activities of dithranol.[1] Reactive oxidants, especially H_2O_2 and hydroxyl radicals, are potent anti-proliferative and immunosuppressive agents, which inhibit phagocyte chemotaxis,[2,3] immunoglobulin[4] and interleukin-2[5] production as well as the cytotoxic activity of natural killer cells[4] and the mitogen-induced transformation of B and T lymphocytes.[4,6,7] We have previously

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reported that low concentrations (0·1–1 μg/ml) of dithranol, but not the auto-oxidised form, interact pro-oxidatively with human neutrophils by priming these cells to generate increased amounts of potentially immunosuppressive reactive oxidants during stimulation with the leucotactic tripeptide FMLP.[8] Pro-oxidative interactions of dithranol with neutrophils could also contribute to therapeutic activity by increasing the generation of anti-proliferative, phagocyte-derived oxidants.

In the present study, the pro-oxidative effects of higher dithranol concentrations (> 1 μg/ml) and the stimulus specificity of the priming interactions of dithranol with human neutrophils have been investigated. Measurements of oxygen consumption and luminol-enhanced chemiluminescence (LECL) have been used, since dithranol, which is a reducing agent, interferes with assays of superoxide and H_2O_2 generation.

METHODS

Chemicals and reagents

All chemicals were purchased from the Sigma Chemical Company (St Louis, Mo, U.S.A.) unless indicated. Dithranol was dissolved in dimethyl sulphoxide (DMSO) to give a stock solution of 10 mg/ml. All subsequent dilutions were made in DMSO, and 10 μl dithranol, or DMSO solvent control, added to 1 ml of neutrophil suspensions to give final concentrations of 0·1–60 μg/ml and 1% of dithranol and DMSO respectively. Indicator-free Hanks balanced salt solution (HBSS), pH 7·4, was used as the cell suspending medium for the various assays of neutrophil membrane-associated oxidative metabolism.

Neutrophil preparation

Neutrophils from venous blood taken from healthy adult human volunteers and two individuals with the autosomal recessive form of chronic granulomatous disease (CGD) were prepared as described previously.[8]

Luminol-enhanced chemiluminescence (LECL)

Neutrophils (5×10^5) were incubated in polypropylene vials with 0·1 mM luminol (5-amino-2, 3-dihydro-1·4-phthalazinedione) in 1 ml HBSS at 37°C for 15 min, followed by the addition of 10 μl dithranol (0·1–60 μg/ml) or DMSO solvent control. The LECL responses of the dithranol/DMSO-treated neutropils from control subjects and individuals with CGD were measured using an LKB Wallac (Turku, Finland) luminometer (Model 1251). LECL readings were integrated for 5 s intervals and plotted as millivolts per second ($mV\,s^{-1}$). To exclude neutrophil-independent interactions of dithranol with the assay system two control systems were included: (i) a cell-free system containing dithranol and luminol and (ii) neutrophils maintained at 4°, 22° and 37°C during exposure to dithranol (40 μg/ml).

The effects of catalase, 25 units/ml final concentration, and of the protein kinase C inhibitor H-7[9] (100 μM, 1-[5-isoquinolinesulphonyl]-2-methylpiperazine) on the LECL responses of neutrophils co-incubated with 20 and 40 μg/ml were also investigated. Both agents were present during the 15 min pre-incubation period of neutrophils at 37°C. In a single experiment the LECL responses of neutrophils activated with 20 μg/ml dithranol were compared with neutrophils activated with the following stimuli at pre-determined optimal concentrations: (i) the synthetic chemotactic tripeptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP; 1 μM), (ii) the calcium ionophore A23187 (1 μM), (iii) opsonized zymosan (1 mg/ml) and (iv) the tumour promotor phorbol 12-myristate 13-acetate (PMA; 10 ng/ml).

The priming effects of dithranol on the LECL responses of neutrophils activated with calcium ionophore, opsonized zymosan and PMA were also investigated. Dithranol (0·1–5 μg/ml) was added to neutrophils 1 min prior to activation with the three different stimuli and LECL recorded as described.

Oxygen consumption by neutrophils

This was measured by use of an oxygen electrode (Model DW1, Hansatech Ltd, King's Lynn, Norfolk, U.K.). Neutrophils (2×10^7) in 3 ml of HBSS were pre-incubated at 37°C for 15 min. Baseline oxygen consumption was measured for 5 min, followed by addition of 30 μl of dithranol (20 and 40 μg/ml) or DMSO control. Oxygen consumption was measured for an additional 8–12 min during the period when the reduction in PO_2 was linear. Neutrophil-free systems were also included to control for oxygen consumption due to auto-oxidation of dithranol. These results are expressed as nmol O_2 utilized/5 min/2×10^7 cells.

Viability studies

Neutrophils maintained at 22°C and 37°C were coincubated with DMSO, dithranol (10 and 40 μg/ml) and PMA (10 ng/ml) for 15 min at 37°C, transferred to an ice-bath and viability measured by eosin (0·1%) exclusion. PMA was included to control for the effects of auto-toxic phagocyte derived oxidants. Incubation at 22°C was included to partly dissociate potential toxicity due to products of dithranol auto-oxidation from auto-toxic products generated by dithranol-activated neutrophils.

Expression and statistical analysis of results

The results of each series of experiments are presented as the mean values ± the standard error of the mean (SEM). Results obtained in dithranol-treated systems were compared with the corresponding untreated controls for each experiment using the Student's paired *t*-test.

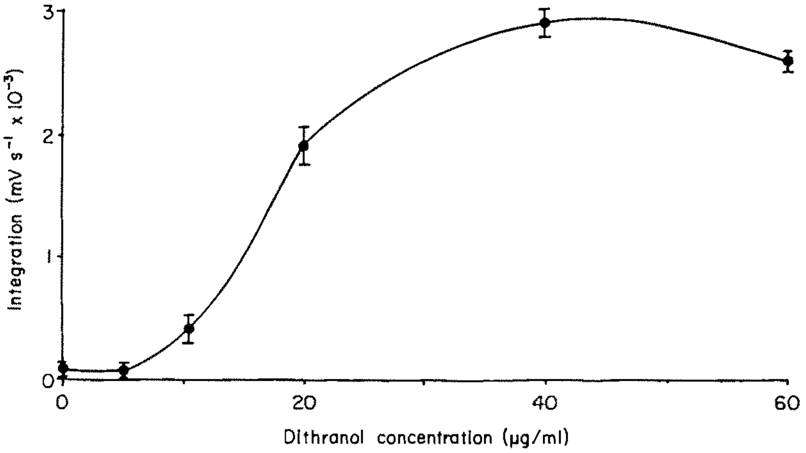

FIGURE 1. The effects of varying concentrations of dithranol (5–60 μg/ml) on the peak LECL responses of human neutrophils. The results of four experiments using neutrophils from two different donors are presented as the mean peak LECL values, reached within 3–5 min ± the SEM's as mV s^{-1}.

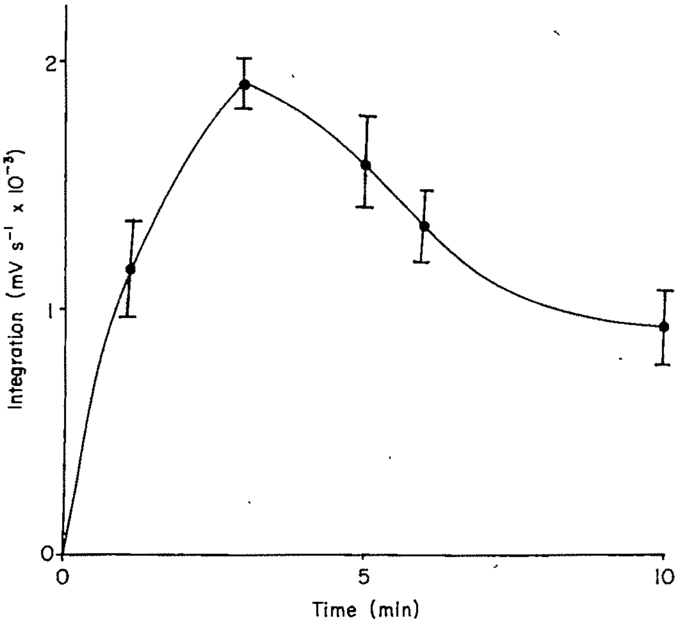

FIGURE 2. The kinetics of dithranol (20 μg/ml)-mediated activation of LECL of human neutrophils. The results of a single representative experiment are expressed as the mean values of triplicate determinations as mV s^{-1}. Similar results to those shown here were observed in a total of seven different experiments.

RESULTS

Effects of dithranol on LECL

These results are shown in Figure 1. Dithranol caused a dose-related activation of LECL which was statistically significant at concentrations of 10 μg/ml and greater and maximal at 40 μg/ml. The kinetics of LECL of neutrophils activated with 20 μg/ml dithranol are shown in Figure 2. Dithranol caused a prompt activation of neutrophil LECL which peaked at 3–4 min, then subsided. Dithranol at all concentrations tested did not activate LECL in the absence of neutrophils. Likewise dithranol (20 μg/ml) did not activate LECL when neutrophils from individuals with CGD were used. The background LECL values for CGD neutrophils in the absence of dithranol were 5 ± 1 and 4 ± 1 mV s^{-1} for each individual, whilst the corresponding values for dithranol-treated CGD neutrophils were 14 ± 1 and 12 ± 1 mV s^{-1}. Further evidence to support the involvement of neutrophil membrane-associated oxidative metabolism was obtained from temperature experiments. The background, unstimulated LECL responses of control neutrophils incubated at 4, 22 and 37°C were 21 ± 4, 64 ± 3 and 143 ± 12 mV s^{-1} respectively; the corresponding peak LECL responses of dithranol (40 μg/ml)-treated neutrophils were 30 ± 2, 361 ± 67 and 2943 ± 129 mV s^{-1} respectively (mean value ± SEM of five replicates from a single experiment).

Data from three experiments undertaken to compare the pro-oxidative potency of 20 μg/ml dithranol relative to various standard stimuli (1 μM FMLP and calcium ionophore, 10 ng/ml PMA and 1 mg/ml opsonized zymosan) are shown in Table 1.

TABLE 1. Comparison of the peak LECL responses of neutrophils activated with dithranol, FMLP, calcium ionophore, opsonized zymosan and PMA

Test-system	LECL
Control, dithranol-free neutrophils	36 ± 7*
Neutrophils + 20 μg/ml dithranol	1651 ± 136
Neutrophils + 1 μM FMLP	503 ± 85
Neutrophils + 1 μM calcium ionophore	784 ± 181
Neutrophils + 1 mg/ml opsonised zymosan	2367 ± 164
Neutrophils + 10 ng/ml PMA	1934 ± 164

* The results of three experiments using neutrophils from different donors are expressed as the mean peak LECL values ± the SEMS's as mV s^{-1}.

Catalase at a concentration of 25 u almost totally inhibited the LECL responses of dithranol-treated neutrophils. The LECL values for unstimulated neutrophils and for cells stimulated with 20 μg/ml dithranol in the absence and presence of catalase were 48 ± 5, 1556 ± 105 and 205 ± 60 mV s^{-1} respectively (results of three different experiments). H-7 also inhibited the LECL responses of dithranol-activated neutrophils and these results are shown in Table 2.

The priming effects of low-concentrations of dithranol (0·1–2·5 μg/ml) are shown in Table 3. These were maximal at 1 μg/ml with calcium ionophore and at 2·5 μg/ml with opsonised zymosan. However the LECL responses of neutrophils activated by PMA were slightly inhibited by dithranol.

Auto-oxidized dithranol at concentrations of 0·1–2·5 μg/ml neither primed nor activated LECL responses of neutrophils (not shown). However higher concentrations activated LECL, but to a lesser extent than the reduced form. The LECL values for control neutrophils and cells treated with 20 μg/ml of reduced and auto-oxidized dithranol were 51 ± 3, 1431 ± 30 and 486 ± 77 mV s^{-1} respectively (data from three different experiments).

TABLE 2. Effects of H-7 on the dithranol-activated peak LECL responses of human neutrophils

Test-system	LECL
(a) Control, dithranol-free neutrophils	82 ± 2*
(b) Neutrophils + 20 μg/ml dithranol	1824 ± 294
(c) Neutrophils + 20 μg/ml dithranol + 100 μM H-7	994 ± 124†
(d) Neutrophils + 40 μg/ml dithranol	3056 ± 346
(e) Neutrophils + 40 μg/ml dithranol + 100 μM H-7	2024 ± 156‡

* The results of 6 experiments using neutrophils from three different donors are expressed as the mean peak LECL values (reached within 3–5 minutes) ± the SEMS's as mV s^{-1}.

† $P < 0{\cdot}01$ and ‡ $P < 0{\cdot}005$ for comparison of systems b with c, and d with e respectively.

TABLE 3. Measurement of the effects of low concentrations of dithranol on the peak LECL responses of neutrophils activated with calcium ionophore, PMA and opsonized zymosan

Test-system	LECL
(a) Neutrophils only	27 ± 11*
(b) Neutrophils + 1 μg/ml dithranol	26 ± 10
(c) Neutrophils + 2·5 μg/ml dithranol	20 ± 7
(d) Neutrophils + 1 μM calcium ionophore	430 ± 62
(e) Neutrophils + 1 μg/ml dithranol + 1 μM calcium ionophore	669 ± 138†
(f) Neutrophils + 10 μg/ml PMA	1230 ± 163
(g) Neutrophils + 1 μg/ml dithranol + 10 ng/ml PMA	1011 ± 88
(h) Neutrophils + 1 mg/ml opsonized zymosan	1416 ± 381
(i) Neutrophils + 2·5 μg/ml dithranol + 1 mg/ml opsonised zymosan	1789 ± 326‡

* The results of 4–5 experiments using neutrophils from different donors are expressed as the mean peak LECL values ± the SEMS's as mV s^{-1}.

† $P < 0{\cdot}025$ and ‡$P < 0{\cdot}005$ for comparison of systems d with e, and for h with i respectively.

Effects of dithranol on oxygen consumption by neutrophils

Dithranol activated oxygen uptake by neutrophils, and at concentrations of 20 and 40 μg/ml the respective values were 11 ± 1 and 24 ± 4 nmol O_2/5 min/2×10^7 neutrophils (mean values ± SEM's of three different experiments). In cell-free systems dithranol *per se* did not cause detectable oxygen consumption. Likewise basal consumption of untreated neutrophils was neglible.

Neutrophil viability

Neutrophil viability was 92 ± 3% and was not altered by a 15 min exposure of the cells to 10 and 40 μg/ml dithranol or 10 ng/ml PMA.

DISCUSSION

The results show that dithranol mediates dose-related priming and activation of the LECL responses of human neutrophils. Low dose priming and high dose activation have been described for other stimulants of neutrophil membrane-associated oxidative metabolism such as FMLP[10,11] and diacylglycerols.[12] The priming effects of low concentrations of dithranol (0·1–1 μg/ml) on the LECL responses of FMLP-activated neutrophils have been previously described,[8] and in the present investigation similar effects were observed with calcium ionophore and opsonized zymosan. However, primed stimulation was not observed when neutrophils were activated with PMA. Activation of LECL was observed with dithranol at concentrations of 10 μg/ml and greater, with maximal activity at 40 μg/ml. Dithranol at these concentrations also increased oxygen consumption by neutrophils. LECL activation was temperature-dependent and required intact cellular membrane-associated oxidative metabolism as evidenced by markedly diminished responses at 4° and 22°C, and also when neutrophils from individuals with CGD were used. This demonstrates that the LECL originates from oxidants generated by dithranol-activated neutrophils, and not from dithranol auto-oxidation products. At a dithranol concentration of 20 μg/ml the LECL responses are greater than those initiated by 1 μM FMLP and calcium ionophore, but somewhat less than PMA and opsonized zymosan. The inhibitory effects of catalase on dithranol-activated LECL responses of neutrophils demonstrate the involvement of neutrophil-derived H_2O_2. Inhibition was also observed with H-7, which supports previous findings that the pro-oxidative interactions of dithranol with human neutrophils involve activation of protein kinase C.[8]

Protein kinase C is a calcium and phospholipid dependent, cytosolic enzyme[13] which is utilized by some stimuli of neutrophil membrane-associated oxidative metabolism,[14] such as diacylglycerols,[15] PMA[16] and benoxaprofen,[17] to activate NADPH-oxidase, the oxidant generating enzyme of phagocytes.[18] Diacylglycerols, released from membrane phosphoinositides by the action of phospholipase C, are the stereospecific, physiological activators of protein kinase C.[19] Little specificity appears to be directed towards the fatty acyl chains of the diglycerides and as long as the diacyl chains are suitably hydrophobic to permit insertion in the bilayer cell membrane, an active diglyceride will result.[19] The specificity is directed toward the glycerol backbone and hydrophilic oxygen moieties of the diglyceride. The removal of one or more of these (oxygen) atoms leads to almost total loss of biological activity.[19] Despite substantial structural differences, the dithranol molecule shares some structural features with diacylglycerols, which may facilitate interactions with protein kinase C. These are: an apparent 'polarity' with distinct hydrophobic and hydrophilic moieties,[20] which would facilitate interactions with the cell membrane and protein kinase C respectively, and a strategically situated triad of oxygen atoms at the hydrophilic side of the molecule, which is an apparent requirement for optimal activation of the enzyme.[19] It has previously been reported that dithranol activates protein kinase C, by apparent substitution for diacylglycerol.[8] This has been confirmed in the present study in experiments with H-7. Auto-oxidation of dithranol also leads to loss of 'polarity'[20] and the generation of auto-oxidation products which are therapeutically inactive[20] and which do not activate protein kinase C or interact pro-oxidatively with neutrophils.[8] However it has been reported that dithranol and chrysarobin do not interfere with the binding of phorbol esters with receptors, presumably diaclyglycerol binding sites on protein kinase C, on mouse epidermal cells.[21,22] This is not necessarily in conflict with the present report, since distinct binding sites for phorbol esters and diacylglycerols have been implicated.[23] Alternatively interference with phorbol ester binding to protein kinase C may be difficult to detect due to the rapid, auto-oxidative inactivation of dithranol, especially at the low concentrations used (1–3 μM).[21,22]

In conclusion, dithranol may prime or directly activate oxidant generation by human neutrophils in inflammatory infiltrates leading to the release of the potent anti-proliferative, immunosuppressive agent, H_2O_2[5,6] This phagocyte-derived oxidant is a potential mediator of both the therapeutic and irritant activities of dithranol. However, dithranol is a highly reactive agent and therapeutic activity may involve various interacting mechanisms,[1] which affect different cell types[24,26] and enzyme systems.[27,28] Since the therapeutic concentrations of dithranol in the skin range from 1–100 μM[29] (0·2–22 μg/ml) it is probable that both the priming and stimulating interactions of this agent with human phagocytes occur *in vivo*.

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Flow cytometric analysis and sorting of HLA-DR$^+$CD1$^+$ Langerhans cells

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SUMMARY

There is a considerable need for reliable methods for enumeration and enrichment of Langerhans cells (LCs), since they continue to be the subject of intensive investigation in normal and diseased skin. It has been claimed that standard labelling with either anti-HLA-DR or OKT6 antibodies alone may fail to identify potentially important subsets of LCs with the phenotypes HLA-DR$^+$CD1$^-$ and HLA-DR$^-$CD1$^+$. We report here on flow cytometric analysis of suction blister-derived normal epidermal cell (EC) suspensions, double stained with phycoerythrin-conjugated anti-HLA-DR and fluoresceinated OKT6. In seven separate experiments, no evidence for the existence of either HLA-DR$^+$CD1$^-$ or HLA-DR$^-$CDI$^+$ ECs was obtained. We found that HLA-DR$^+$CD1$^+$ LCs, which constituted a mean of 2·5% (±0·3 SEM) of all ECs, could be readily identified on the basis of fluorescence, and that their light scatter characteristics were those of moderately sized cells of low granularity. We further describe our method for flow cytometric enrichment of such HLA-DR$^+$CD1$^+$ LCs for functional studies, based on selection on both fluorescence and light scatter criteria. Enrichment is to greater than 90% purity, and the method is applicable to the small number of ECs (approximately 1×10^6) obtained from a suction blister.

Bone marrow-derived dendritic epidermal Langerhans cells (LCs) constitute about 2% of all epidermal cells (ECs), and are defined ultrastructurally by a lobate nucleus, a clear cytoplasm devoid of tonofilaments, desmosomes or melanosomes, and by the presence of characteristic cytoplasmic granules.[1] Cells with most of these ultrastructural features but which lack Langerhans cell granules have been termed indeterminate cells. LCs are thought to play a crucial role in contact sensitization and in cutaneous immunosurveillance against viral infection and neoplasia.[2–5] LCs are also thought to be involved in the pathogenesis of a number of other important lymphocyte-mediated skin disorders, including atopic dermatitis, lichenoid eruptions, mycosis fungoides, and graft vs. host disease, and may harbour the HIV-1 virus in the acquired immunodeficiency syndrome.[2–5] Furthermore, the therapeutic action of topical corticosteroids, psoralen plus long-wave ultraviolet light (PUVA), and X-irradiation may be

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partly related to their effects on LCs.[4–8] For these reasons, analysis of LC numbers and function continues to be a central part of many studies in dermatological research.

With the exception of acrosyringeal keratinocytes, LCs and indeterminate cells are the only cells in normal mammalian epidermis which express surface Ia (immune response-associated) or class II major histocompatibility complex (MHC) antigens (HLA-DR in man).[2–5] Human LCs also express CD1 surface antigen recognised by OKT6 monoclonal antibody.[9,10] It has been claimed that CD1 is a better marker in human skin sections, and that anti-class II MHC antibodies may significantly underestimate the number of LCs.[10] However, others have reported that OKT6 reactivity is difficult to analyze objectively in cryostat sections because of the variability in staining of LC dendrites.[11] Some studies have reported a slight preponderance of CD1$^+$ over HLA-DR$^+$ECs in EC suspensions.[12] Conversely, in other studies in which EC suspensions were double stained with anti-HLA-DR and OKT6, HLA-DR$^+$ ECs exceeded CD1$^+$ECs. Vaigot and co-workers identified two subpopulations of LCs (90% were HLA-DR$^+$CD1$^+$, and 10% were HLA-DR$^+$CD1$^-$),[13] whilst Dezutter-Dambuyant and co-workers found three subpopulations of positively stained ECs (97·3% were HLA-DR$^+$CD1$^+$, 2·2% were HLA-DR$^+$CD1$^-$, and 0·5% were HLA-DR$^-$CD1$^+$).[14] Some authors have reported that there is heterogeneity among HLA-DR$^+$ ECs: the 25% which are strongly HLA-DR$^+$ include indeterminate cells as well as some LCs, whilst the remaining 75% which are only weakly HLA-DR$^+$ include the majority of typical LCs.[15,16] Since labelling with anti-HLA-DR antibodies and/or OKT6 has become one of the standard methods for enumeration and enrichment of LCs, one of the aims of this study was to investigate the controversial existence of HLA-DR$^+$CD1$^-$ and HLA-DR$^-$CD1$^+$ subsets of LCs and/or indeterminate cells. This issue has attracted renewed attention with reports that subgroups of CD1$^+$ ECs may express products of T cell receptor γ/δ-encoding genes, analagous to the murine dendritic Thy-1$^+$EC.[17,18]

There is an increasing need to obtain purified populations of LCs in isolation in order to further our knowledge of LC biology, especially since keratinocytes have been shown to secrete a wide variety of cytokines which may either boost or suppress LC-dependent immunological responses in the skin.[19] Methods for moderate enrichment of LCs include Fc-receptor rosetting, density gradient centrifugation, and solid-phase immunoabsorption (panning) using monoclonal antibodies or *Staphylococcus aureus* treated cells. Flow cytometry has been reported to be a superior method for LC enrichment in rodent skin with yields of up to 98% purity.[20–22] Flow cytometry has also been applied to analysis and enrichment of LCs in normal human skin;[12,13,20,23–25] where stated, enrichment has ranged between 60% and 83%.[20,23,25] We report here on flow cytometric analysis of suction blister-derived normal EC suspensions double stained with anti-HLA-DR and OKT6. We further describe our sorting procedure which enables collection of sufficient purified HLA-DR$^+$CD1$^+$LCs from a suction blister to enable functional studies to be carried out.

METHODS

Subjects and treatment

Epidermal sheets free of dermal contamination were obtained from normal forearm skin of volunteers by the suction blister method as previously described.[6,24] Informed consent was obtained from all individuals.

Preparation of cells

Disaggregated epidermal cell (EC) suspensions were obtained following incubation of suction blister roofs with 0·5% trypsin (U.S. Biochemical Co., Cleveland, OH, U.S.A.) in Dulbecco's phosphate buffered saline (PBS, Gibco, Paisley, Scotland, U.K.) as previously described.[6] The viability of ECs as determined by trypan blue exclusion ranged between 90 and 95%.

Immunofluorescence staining

EC suspensions were double stained by a one step immunofluorescence method as previously described.[7,8,24] Fluoresceinated OKT6 (Ortho Diagnostic Systems Inc., High Wycombe, Bucks, U.K.) and phycoerythrin-conjugated anti-HLA-DR (Lab Impex Ltd., Teddington, Middx., U.K.) were combined in optimal dilutions (1:20) in PBS, and incubated with the cells for 30 min at 4°C. The epidermal cells were then washed three times in PBS. The viability of ECs as determined by trypan blue exclusion after immunostaining ranged between 90 and 95%. For preliminary experiments, propidium iodide (PI) at a final concentration of 10 μg/ml was added to cell suspensions during the final wash, to identify dead cells.

Flow cytometric analysis and sorting

Flow cytometric analysis was performed on EC suspensions which had been stained as above. ECs were resuspended in medium consisting of RPMI 1640 (Gibco UK Ltd.) with added antibotics, 2 mM L-glutamine and 15% human AB serum (Sigma Chemical Co., Poole, Dorset, U.K.) (complete medium), and passed through a sterile nylon mesh filter. The EC suspensions were analysed using a Becton Dickinson FACStar equipped with a 5 W argon ion laser used at an excitation wavelength of 488 nm and a power of 200 mW. A threshold was set on forward angle light scatter signals to exclude particulate debris. Cells were enumerated relative to green fluorescence, red fluorescence, forward scatter, and side (right angle) light scatter. Ten thousand events of list mode correlated data were acquired and analysed using a Hewlett Packard computer and the Consort 30 data analysis program.

EC suspensions were sorted at a rate of approximately 1,000 cells/sec to obtain HLA-DR$^+$CD1$^+$ and HLA-DR$^-$CD1$^-$ cell populations. Sorting gates were set on both fluorescence and scatter signals to exclude particulate debris and clumped cells which re-accumulated on settling of the EC suspension after filtration. The sorting procedure was monitored by examining aliquots of the sorted populations, mounted on glass slides under a coverslip, using a Leitz EB22 fluorescence microscope. There was necessarily a considerable loss of ECs during washes following staining, and during calibration of the flow cytometer; in addition, a proportion of each EC suspension was used for flow cytometric analysis. Not surprisingly therefore, the yield, after sorting, of HLA-DR$^+$CD1$^+$ LCs available for functional studies was also low, ranging between 1×10^3 and 3×10^3 from a single suction blister.

RESULTS

Propidium iodide (PI) staining of EC suspensions

Figure 1 shows a representative experiment in which the two colour fluorescence contour graph of ECs stained with PE-DR and FITC-OKT6, as well as PI, is displayed. There is a readily identifiable population of viable HLA-DR$^+$CD1$^+$ LCs (represented in box 1), which constituted a mean of 1·88% ($\pm$0·03 SEM, $n=2$) of all ECs, and there are no HLA-DR$^-$CD1$^+$ECs. The absence of HLA-DR$^-$CD1$^+$ECs was confirmed in a second experiment in which PI was added, as well as in further experiments without PI addition (see below). A number of ECs take up red stain alone (represented in box 2); it is not possible to distinguish between ECs which might be HLA-DR$^+$CD1$^-$, and dead cells which have taken up PI. In two

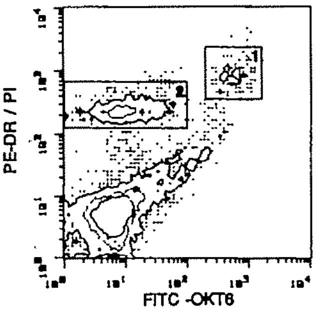

FIGURE 1. Two-colour flow cytometry of ECs stained with phycoerythrin-conjugated anti-HLA-DR (PE-DR), propidium iodide (PI) and fluoresceinated OKT6 (FITC-T6); representative experiment (10,000 events analysed). The graph is a contour plot in which individual dots represent a minimum of one event, and concentric contour lines represent increasing numbers of events. Viable HLA-DR^+CD1^+ LCs are represented within box 1, and ECs which have taken up PI (and/or PE-DR alone) are shown within box 2. Note the absence of HLA-DR^-CD1^+ ECs.

experiments, a mean of 21·6% (±9·3 SEM) of ECs stained with PI and/or PE-DR alone. The relatively high number of dead cells in these preparations may reflect the fact that PI is progressively toxic to ECs with time. Our aim was to develop a method to enable comparison of functional properties of LCs from EC suspensions from uninvolved and involved, or treated and untreated, skin. Since such EC suspensions would normally be processed in parallel, given the inevitable turnaround time for calibration of the flow cytometer and for sorting, artefactual differences could arise because populations of ECs had been exposed to PI for different times. For this reason, and also because we wished to be able to identify any HLA-DR^+CD1^-ECs which might be present, the PI staining step was omitted in further experiments.

Flow cytometric characteristics of Langerhans cells

A representative contour plot of ungated forward and side light scatter signals of an EC suspension is shown in Figure 2a. Forward scatter principally relates to particle or cell size, whereas side scatter relates to properties of cells such as granularity, density and smoothness of membrane.[26] Signals in the low forward scatter range seen here are, therefore, derived from particulate debris, while signals in the very high forward or side scatter range are derived from clumps of cells, which escaped the filtration procedure or formed on settling of the EC suspension. Figure 2b shows the corresponding contour plot illustrating the intensity distribution of ungated red and green channel fluorescence signals, on staining of EC suspensions with PE-DR and FITC-T6. The HLA-DR^+CD1^+ LC population is represented in box 1; the mean percentage of such HLA-DR^+CD1^+ LCs in ungated EC suspensions was 2·50% (±0·30 SEM, $n=5$; Table 1). Neither HLA-DR^+CD1^- nor HLA-DR^-CD1^+ ECs appear to be present; such ECs were consistently absent in a further four experiments.

Figure 2c shows the result of gating on the HLA-DR^+CD1^+ LCs represented in Figure 2b (box 1), and redisplaying their correlated light scatter contour distribution. Most of the light scatter signals from the HLA-DR^+CD1^+ LCs are grouped together at a position on the contour plot indicating cells of moderate size (intermediate forward scatter) and low granularity (low side scatter). In addition, however, there are some signals in the high forward and/or side scatter regions (arrows), suggestive of LCs associated with other cells in clumps. Therefore, in order to provide highly purified populations of LCs in single cell suspension for functional studies, it is necessary to apply gates to exclude such clumped cells during the sorting procedure.

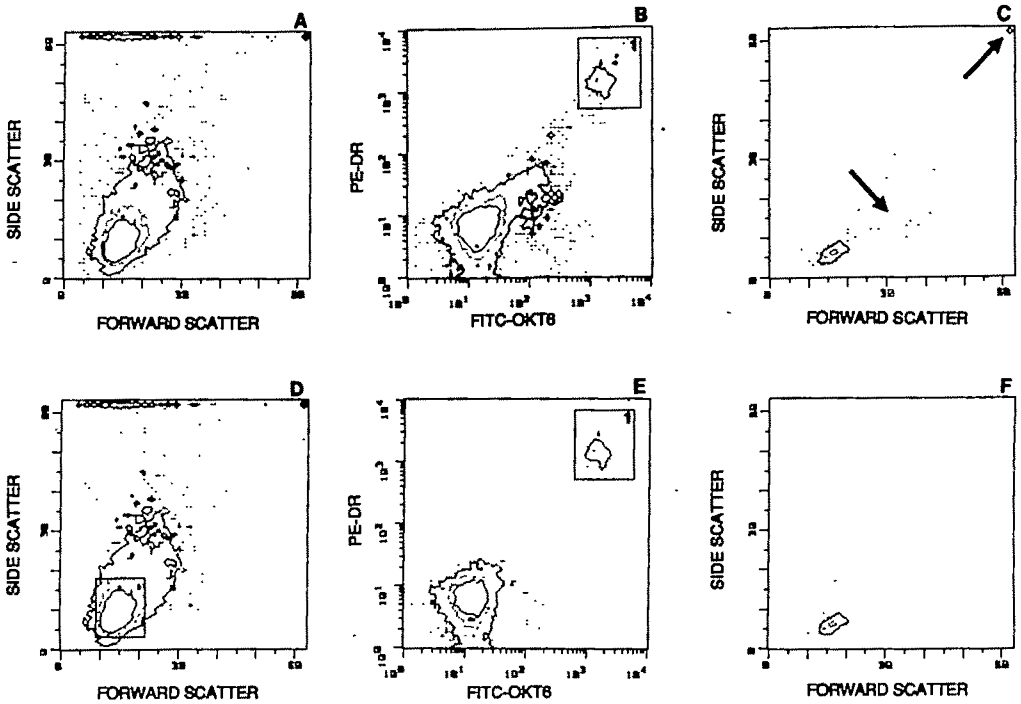

FIGURE 2. Flow cytometric analysis of EC suspension stained with phycoerythrin-conjugated anti-HLA-DR (PE-DR) and fluoresceinated OKT6 (FITC-T6); representative experiment (10,000 events analysed). (a). Contour plot showing ungated forward and side light scatter signals from the EC suspension. Signals in the low forward scatter range represent particulate debris, and signals in the high forward and/or side scatter regions represent clumps of cells. (b). Contour plot showing the ungated fluorescence intensity distribution of the EC suspension stained with PE-DR and FITC-T6. Box 1 indicates HLA-DR$^+$CD1$^+$ LCs which constitute 3·66% of all ECs; note the absence of HLA-DR$^+$CD1$^-$ and HLA-DR$^-$CD1$^+$ ECs. (c). Contour plot showing the correlated forward and side light scatter signals of the HLA-DR$^+$CD1$^+$ LCs represented in box 1 in (b). Note the presence of signals in the high forward and/or side scatter regions (arrows), suggestive of LCs associated with other cells in clumps. (d). The box, which indicates the position of the gates applied on forward and side light scatter signals to exclude particulate material and clumped cells, contains 71·7% of total events. (e). Contour plot showing the fluorescence intensity distribution of the EC suspension stained with PE-DR and FITC-T6, after gating on scatter signals as shown in the box in (d); single HLA-DR$^+$CD1$^+$ LCs are represented in box 1. (f). Correlated light scatter contour plot of the HLA-DR$^+$CD1$^+$ LCs represented within box 1 in (e); note relatively homogeneous population of cells of moderate size (intermediate forward scatter) and low granularity (low side scatter).

Gating procedures for FACS analysis and sorting of Langerhans cells

Application of a gate on forward and side light scatter signals from the EC suspension, as shown by the box in Figure 2d, excludes particulate debris and clumped cells, yet retains the majority of single ECs. Gating on light scatter signals prior to analysis did not significantly ($P = 0{\cdot}5$, Student's t-test) alter the proportion of HLA-DR$^+$CD1$^+$ LCs in EC suspensions (Table 1). Figure 2e shows the contour plot of red and green fluorescence intensities of the EC suspensions stained with PE-DR and FITC-T6, after selecting data by application of such gates on scatter signals; the population of single HLA-DR$^+$CD1$^+$ LCs is represented in box 1. Figure 2f shows

TABLE 1. Flow cytometric enumeration of HLA-DR$^+$CD1$^+$ Langerhans cells in EC suspensions before and after gating on light scatter characteristics

	Percentage of HLA-DR$^+$CD1$^+$LCs	
Experiment	% of Total (Ungated) ECs	% of Gated ECs*
1	3·66	3·66
2	2·10	2·46
3	2·08	1·84
4	2·18	1·96
5	2·46	2·68
Mean ± SEM	2·50 ± 0·30	2·52 ± 0·32

* EC suspensions were gated on forward and side angle light scatter to eliminate particulate debris and clumped cells.

the result of gating on these single HLA-DR$^+$CD1$^+$ LCs and redisplaying their correlated light scatter contour plots. The remaining single LCs are seen as a relatively homogeneous population of moderately sized cells of low granularity. LCs were similarly characterized in terms of light scatter in six further experiments.

For sorting of single HLA-DR$^+$CD1$^+$ LCs, we defined four parameter sort windows; on forward and side light scatter parameters (similar to the box in Fig. 2d), and on red and green fluorescence parameters (similar to box 1 in Fig. 2e). Combined direct and fluorescence microscopy of populations enriched for HLA-DR$^+$CD1$^+$ LCs in this manner showed that they contained 90·9% ± 2·7 of cells staining with both fluorochromes (mean ± SEM, $n = 3$), and that the negatively stained ECs were always closely adherent to an adjacent HLA-DR$^+$CD1$^+$ LC; adherence was confirmed by the fact that the cells could not be separated within the fluid suspension by pressure on the coverslip. Less than 1% of cells in populations enriched for HLA-DR$^-$CD1$^-$ cells were HLA-DR$^+$CD1$^+$.

DISCUSSION

Langerhans cells were readily identified on flow cytometric analysis of EC suspensions double stained with PE-DR and FITC-OKT6 as HLA-DR$^+$CD1$^+$ ECs, with light scatter characteristics indicating that that they were of moderate size but lower granularity than keratinocytes.[26] This was in keeping with their ultrastructural appearance as clear cells devoid of intermediate filaments.[1] In five separate experiments using suction blister-derived EC suspensions, we did not observe either HLA-DR$^+$CD1$^-$ or HLA-DR$^-$CD1$^+$ ECs, in contrast to previous reports.[12–16] These previous studies used relatively large amounts of skin removed from cadavers, at surgery or by keratome, with subsequent processing involving trypsinisation of whole skin specimens. This procedure could have led to contamination of EC suspensions by non-LC HLA-DR$^+$ dermal monocyte-macrophages. In the study reporting the presence of 10% HLA-DR$^+$CD1$^-$ ECs, it was stated that additional ECs were released into the medium by agitation of the dermis.[13] Pre-enrichment of EC suspensions for leukocyte-like cells by Ficoll–Hypaque density gradient centrifugation[12] could in addition have exaggerated the contribution of such contaminating dermal cells. We found no evidence for the existence of heterogeneity amongst LCs in terms of the surface markers HLA-DR and CD1.

A major aim of this study was to develop a reliable method for producing highly purified populations of LCs in single cell suspension for functional studies *in vitro*, by flow cytometric sorting. A previous report defined human LCs as cells with low right angle (side) scatter but high forward scatter,[12] and advocated sorting of unlabelled EC suspensions for LCs on the basis of light scatter characteristics alone. These authors used preliminary Ficoll–Hypaque density gradient centrifugation of EC suspensions to pre-enrich for LCs; this procedure removes larger keratinocytes and corneocytes. Ficoll–Hypaque density gradient centrifugation has been used by other authors to pre-enrich for murine LCs, since they found difficulty in setting sorting gates with confidence using crude EC suspensions.[22] Indeed, the initial attempts at flow cytometric sorting for LCs based on labelling with a single fluorochrome resulted in 30% to 40% contamination with keratinocytes.[20,23] Because the EC recovery after Ficoll–Hypaque density gradient centrifugation of suction blister-derived EC suspensions is only in the order of 15% to 28%,[24] it is not practicable to use this procedure when constrained by a strictly limited total number of ECs available per experiment.

We have devised a sorting method applicable to the approximately 1 × 10^6 unfractionated ECs obtainable from a single suction blister. Some previous studies have used gating on forward scatter to eliminate large cells and clumps.[23,25] Having established that LCs are HLA-DR$^+$CD1$^+$ ECs with low side, and moderate forward, light scatter characteristics, we gated simultaneously on forward and side scatter, and on green and red fluorescence. Sorting for HLA-DR$^+$CD1$^+$ LCs in this manner yielded highly purified populations of LCs (over 90% HLA-DR$^+$CD1$^+$ ECs). Because of the observed tendency of some LCs in EC suspensions to associate with and adhere to keratinocytes, it may not be possible to exceed this level of purification using current sorting technology. Whilst our method is undoubtedly associated with a low overall yield, it reliably produces sufficient highly purified HLA-DR$^+$CD1$^+$ LCs from a single suction blister to enable functional studies to be carried out. In studies reported elsewhere,[7,8] HLA-DR$^+$CD1$^+$ LCs sorted according to the above protocol were shown to retain their alloantigen presenting function as determined in the allogeneic epidermal lymphocyte reaction. Application of this sorting procedure has enabled us to study the functional properties of the small numbers of residual HLA-DR$^+$CD1$^+$ LCs in human skin following *in vivo* topical corticosteroid or PUVA therapy.[7,8]

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Differential expression of Langerhans cells in the epidermis of patients with leprosy

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SUMMARY

Eighteen patients with lepromatous leprosy (LL) showed a significant reduction ($P < 0{\cdot}001$) of Langerhans cells (LC) irrespective of whether the biopsies were obtained from involved (398 ± 186) or healthy skin (304 ± 98). The cells showed morphological changes consisting mainly of loss of dendritic processes. Twenty-four controls (age, sex and race matched) had a mean number of LC of 632 ± 138. In tuberculoid patients (TT) significant differences were observed, depending on the site of biopsy. Nine biopsies from involved skin had 993 ± 206 LC, whereas 11 from healthy skin had 448 ± 96 ($P < 0{\cdot}001$). This difference was confirmed in six additional borderline tuberculoid (BT) and TT patients in whom biopsies were simultaneouslyy obtained from involved (973 ± 179) and uninvolved skin (498 ± 99). In 10 patients with indeterminate leprosy the LC density did not differ from the control population (630 ± 261). The expression of LC numbers in BT and TT patients may represent migration of these cells from healthy skin to involved areas or mobilization of a central pool. The low density found in LL patients could interfere with adequate presentation of mycobacterial antigens leading to tolerance. Alternatively the presence of T helper cells in TT infiltrates may produce factors that recruit LC; their absence in LL lesions may account for the decrease in LC expression.

Leprosy is produced by the infection with *Mycobacterium leprae* (ML). There is marked variability in the clinical, histological and immunological features of this disease. The tuberculoid polar form (TT) is characterized by well localized skin lesions that histopathologically present organized granulomas containing few if any bacilli. Patients in this group preserve delayed hypersensitivity as evidenced by positive specific skin responses to lepromin (Mitsuda reaction). Study of T lymphocyte subsets in peripheral blood has shown normal or increased helper/suppressor T cells ratios, a phenomenon also observed within the dermal granulomas.

The opposite polar form, lepromatous leprosy (LL), presents with widespread lesions of the skin, mucous membranes and internal organs. On histology there are foamy histiocytes containing viable bacilli, few lymphocytes and giant cells. Cellular immunity in these patients is

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markedly depressed, with anergy to the lepromin test as well as to unrelated antigens. In circulating T cells the helper/suppressor ratio is usually inverted, and lymphocyte blast transformation and response of monocytes to chemotactic stimuli have been reported to be decreased.

Between these two clinical and immunological poles there are two intermediate forms, the indeterminate (I) and borderline types (B). These have distinct clinical, immunological and histopathological features.[1-4]

The differences in bacillary dissemination and clinical expression in leprosy may be due to an alteration in the first phase of the immune response,[5] mainly the presentation of antigens by monocytes, macrophages and dendritic cells. Because the Langerhans cell (LC) is the antigen-presenting cell in the skin, we have quantitated these cells in the epidermis of patients with the various forms of leprosy.

METHODS

Patients

This study involved 30 males and 29 females, aged 14–58 years, from an area in the north-east of Argentina where leprosy is endemic. The patients were classified according to clinical, histological and bacteriological features, as well as their response to the lepromin antigen.[6] Of the 22 tuberculoid patients, 11 were newly diagnosed and untreated, while 11 were undergoing treatment with dapsone. Of the 18 lepromatous patients, seven were untreated and 11 were currently under treatment. All of the four borderline tuberculoid and 10 indeterminate patients were receiving dapsone. The latter group was included in our study although not strictly classified by Ridley and Jopling. The healthy control population consisted of 14 males and 10 females, matched in age, race and nutritional status with the patient population.

Skin biopsies

Shaved-skin biopsy specimens were obtained and were stored at 4°C in citrate buffer, pH 7·0, for no longer than 3 weeks (which was previously determined not to alter LC numbers or morphology). The patients were divided into two groups, in one the biopsies being obtained from lesional skin and in the other from uninvolved skin of the upper back. From two of the tuberculoid (TT) and four of the borderline tuberculoid (BT) patients the specimens were obtained simultaneously from lesional and non-lesional areas. In the healthy population the biopsy samples were from skin on the upper back.

Microscopic enumeration of LC

Each biopsy specimen was bisected and one part was immersed in 2 M sodium bromide solution (Mallinkrodt, Paris, KY, U.S.A.) for 2 h at 37°C. Epidermal sheets were then separated manually from underlying dermis, washed in distilled water for 10 min and fixed in 5% formalin for 20 min. After rinsing in distilled water for 10 min, the epidermal sheets were incubated with adenosine 5′-triphosphate (ATP) (Sigma, St. Louis, MO, U.S.A) for 90 min at 37°C. The specimens were washed and developed in diluted ammonium sulfide (Fisher Scientific, Fair Lawn, NJ, U.S.A.) for 10 min, and mounted with dermal side up in Permount (Fisher Scientific). LC density within each epidermal sheet was determined using a light transmission microscope with a calibrated eye piece (Spencer 10 × 45). For each specimen, the average number of LC per mm^2 of epidermis was counted in one central and four peripheral areas, excluding hair follicles. The reported LC density for each epidermal sheet represents the arithmetic average of the five counts +/− standard error per mm^2 of skin surface.

Four and 6 μm thick vertical sections were cut on a cryostat from the other portion of the biopsy and stained in a similar fashion as for epidermal sheets.[7–9]

OKT6 (CD1$^+$) staining

Six micrometre thick sections were cut with a cryostat at −20°C, air dried for 10 min, fixed in acetone (Fisher Scientific) for 10 min and washed in phosphate buffered saline, pH 7·6 (PBS). The sections were incubated with 5% normal goat serum in PBS, washed in the same buffer and further incubated for 45 min with 100 μl of OKT6 (Ortho Pharmaceuticals, Raritan, NJ, U.S.A.) diluted to 1 μg/ml in PBS containing 3% bovine serum albumin (BSA) (Sigma). Control sections were reacted with non-immune mouse serum. Subsequently the samples were washed in PBS and incubated for 45 min with FITC goat Fab′2 anti-mouse IgG (Tago, Burlingame, CA, U.S.A.) diluted 1 : 10 in PBS-3% BSA. After final washes in PBS the sections were mounted with FA mounting fluid (Difco Laboratories, Detroit, MI, U.S.A.) and examined on an Olympus fluorescence microscope.[10]

RESULTS

Langerhans cells in epidermal sheets of patients presenting with different forms of leprosy were quantitated using ATPase technique (Fig. 1). The epidermis of lepromatous patients showed a marked reduction in the number of LC regardless of whether the biopsy specimen was taken from involved or uninvolved skin (398 ± 186 LC/mm^2 and 304 ± 98, respectively). No statistically significant difference was observed between those groups. Skin samples from the control population were found to have 652 ± 138 LC/mm^2 ($P < 0{\cdot}001$). In contrast, there was a significant difference in the number of LC in patients with tuberculoid leprosy depending on the site of the biopsy. Biopsy specimens from uninvolved skin showed 448 ± 96 LC/mm^2, whereas in lesional skin the number of LC/mm^2 was 993 ± 206 ($P < 0{\cdot}001$). As shown in Figure 1, the LC density of the latter group is significantly higher than in the controls ($P < 0{\cdot}001$). Simultaneous LC counts of lesional and non-lesional skin performed in two patients with tuberculoid leprosy

TABLE 1. Number of Langerhans cells/mm^2 in the healthy and lesional skin of patients with tuberculoid and borderline tuberculoid leprosy

Leprosy type	Healthy skin	Lesional skin
BT	669	1048
BT	383	1266
BT	475	758
BT	513	1008
TT	524	927
TT	427	830
Mean ± SD	498 ± 99	973 ± 179

Biopsies were obtained simultaneously from healthy and diseased skin. A significant difference was observed between both sites ($P < 0{\cdot}001$).

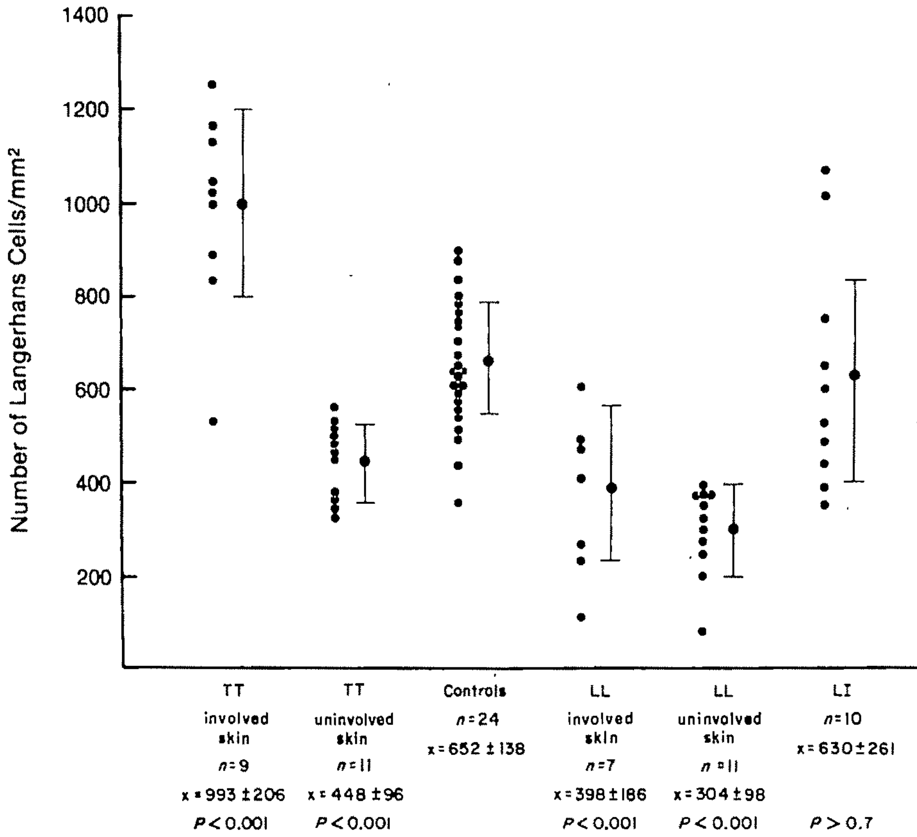

FIGURE 1. Quantitation of Langerhans cells per mm^2 in patients with leprosy. P values represent the difference between the various patient groups and the normal population. The number of LC/mm^2 in the lesional and healthy skin of tuberculoid (TT) patients differed significantly ($P < 0{\cdot}0005$). No difference was found between these two biopsy sites in lepromatous (LL) patients ($P = 0{\cdot}35$).

and four with borderline tuberculoid leprosy showed similar results (973 ± 179 and 498 ± 99 respectively (Table I). In 10 patients with indeterminate leprosy the mean LC/mm^2 of lesion skin (630 ± 261) did not differ from the normal population (Fig. 1).

ATPase activity was localized in the membrane and cytoplasm of LCs; in lepromatous patients there were morphological alterations of LCs, mainly loss of dendritic processes and marked shrinkage of cell bodies (Fig. 2). In vertical sections of skin from normal individuals and patients with LL the distribution of ATPase and CD1$^+$ cells was the same regardless of their number. In tuberculoid patients dendritic cells staining with CD1 and ATP were also seen surrounding the tuberculoid granulomas.

DISCUSSION

It is well established that T cell mediated responses provide effective immunity in leprosy. These responses are intact in the tuberculoid form of the disease (TT), but show a gradual decrease in the lepromatous (LL) pole. In contrast to cells from TT patients, monocytes of

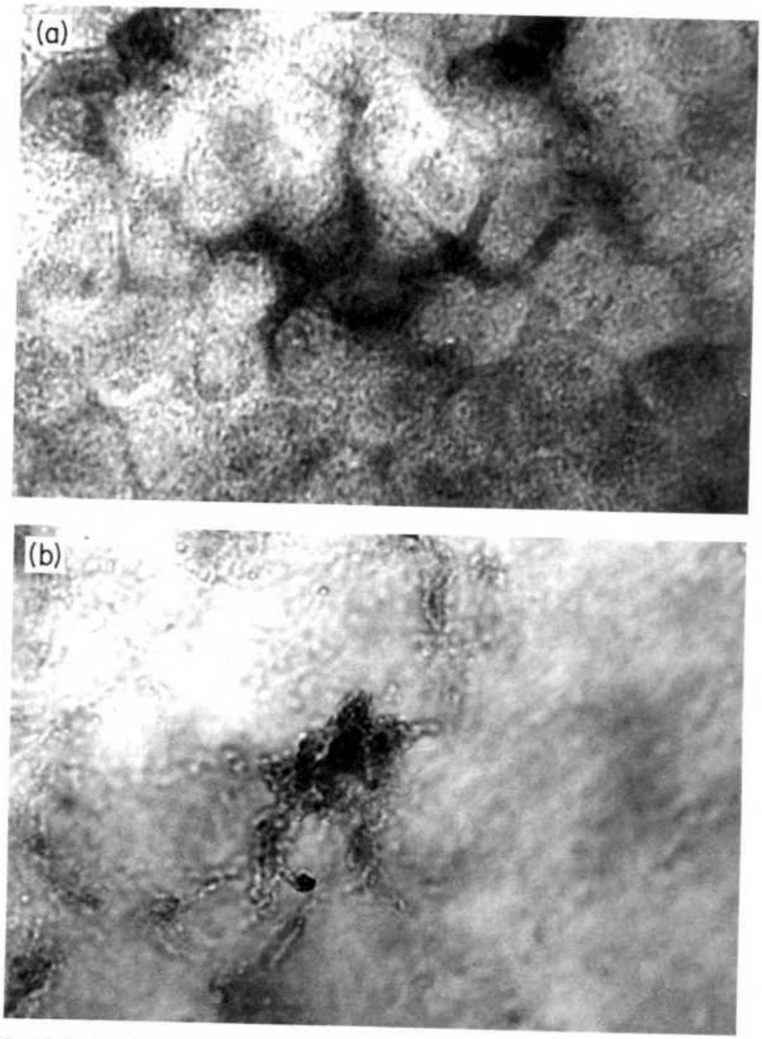

FIGURE 2. Morphological characteristics of LC in epidermal sheets stained with ATP. (a) Normal donor, (b) patient with lepromatous leprosy.

patients with LL have been shown to lack the capacity to be activated in response to the mycobacteria. This defect apparently stems from the lack of production of cytokines, particularly interleukin-2.[5,11–15] It was recently reported that the injection of interferon-γ may reverse cell mediated immune defects in lepromatous leprosy,[16] and repeated injections were associated with a depletion of epidermal LC.[17] Although the cellular response to the infection is markedly depressed in LL, the capacity of these patients to respond to other antigens is only partially impaired, as reflected by the expression of Ia on macrophages.[10]

Langerhans cells have been suggested as participants in the inductive phase of the T cell response to *M. leprae*,[5] and are potent stimulators of T-cell proliferation.[18] These bone marrow derived cells are the only epidermal cells that under normal conditions express Ia/DR antigens and can be differentiated from other cells of the epidermis by the presence of adenosine triphosphatase (ATPase) and the CD1 surface molecules.[19-22]

Since Richter originally described the characteristics of LC in cutaneous leprosy, a number of studies have followed with contradictory results. Cells were reported to be decreased, increased or unchanged in lesional or non-lesional epidermis of patients with lepromatous and/or tuberculoid leprosy.[22-30] We found that in lepromatous patients the density of LCs was markedly reduced in both uninvolved and involved skin, and the morphology of the remaining cells was altered. Patients with the tuberculoid form of leprosy showed an increase in LC density in involved skin and a decrease in uninvolved skin, compared to normal controls. The differences between our results and those of other studies cannot be explained by the techniques used, and whether differences in racial origin of the patients may be a factor remains to be determined.

The host immune response to *M. leprae* may be the major determinant of the clinical manifestations in leprosy.[5] The symptoms of tuberculoid leprosy may reflect an augmented cell mediated immune response expressed by an increased number of Langerhans cells in the epidermis, as well as their presence in granulomas. LCs may generate factors that have the potential to activate macrophages and to produce an influx of inflammatory cells, resulting in the formation of the tuberculoid granuloma. The observation of decreased LCs in uninvolved tuberculoid epidermis, and markedly increased numbers in lesional skin as well as in the dermis could be the result of the migration of these cells from uninvolved skin. Alternatively a central pool of LCs may be mobilized to the dermis and epidermis.[31-33]

Previous studies have shown that there is a distinct difference in T lymphocyte subsets and non-lymphoid mononuclear cell markers[29] between the infiltrates of patients with the lepromatous or tuberculoid form of the disease. In the latter, where we found increased numbers of LCs, the infiltrate is predominantly made up of helper T cells. In lepromatous lesions which have low numbers of LCs, helper T cells are decreased.[28,34] The reduction in numbers of LCs as assessed by ATP staining and expression of CD1 surface marker in these patients may result in lack of stimulation of CD4 cells.[35]

Although it remains unclear whether the alteration in the number of Langerhans cells in the epidermis of patients with leprosy is a primary or secondary phenomenon, investigation into the mechanism of these changes will provide further insight into this disease.

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Identification of cross-reaction patterns in allergic contact dermatitis from topical corticosteroids

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SUMMARY

Contact allergy to topical corticosteroids occurs more frequently than previously supposed. Cross-allergic phenomena are common. On the basis of a review of the literature and our own patch test data on 15 patients, we conclude that positive patchtests to corticosteroids occur approximately six to seven times more frequently in well-defined groups of structurally-related substances than between corticosteroids of different groups. An analogous substitution pattern on the steroid D-ring or the carbon side chain (C_{20}, C_{21}) seems to have a significant influence on the association of positive patchtest results. This is not the case for other structural variables, such as the presence of a double bond in the steroid A-ring or fluoride substitutions on the B-ring. The effect of other factors such as concomitant sensitization and steroid metabolism in the skin on the development of a corticosteroid polyallergy are analysed, and the specificity and sensitivity of cross-allergy phenomena are evaluated. These are important in the selection of a topical steroid in the future treatment of a corticosteroid sensitive patient.

Allergic contact dermatitis from corticosteroids seems to be much more common[1,2] than was previously thought.[3,4] The examination of possible cross-allergy patterns between corticosteroids is essential for the further understanding of the phenomenon of corticosteroid sensitization.[5] This is to obtain information on the molecular structure of the antigen determinant and to develop an optimal patchtest predictor series, as well as to facilitate the therapeutic choice of corticosteroids in sensitized patients.

It needs to be determined whether cross-allergic reactions between corticosteroids occur between all the substances, between some of them, or not at all. In the literature, frequent reference is made to the importance of this differentiation, although no convincing argument has been put forward to substantiate either of these hypotheses.

Several authors have reported multiple positive patchtest results to corticosteroids, and the question arises as to whether they can be interpreted as cross-reactions or whether they are due

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to independent concomitant sensitization. The answer is not simple, because many patients have had a long history of using various topical corticosteroids. In our own patch test series,[2] we were able to demonstrate unambiguous cross-reactions by testing products that were not commercially available in our country and that did not contain common allergenic contaminants.[6] A similar line of argument was recently offered by Sasaki in Japan for budesonide.[7] Cross reactions therefore clearly occur.

In nearly all the case reports on this subject, the number of positive patch test results for corticosteroids is limited. This does not exclude general cross-allergies, since positive results to certain corticosteroids may be regularly missed because of technical inadequacies in the test procedure (inadequate penetration and also a predominant anti-inflammatory effect). In the literature, one generally meets variable clusters of substances to which reactions occur. Furthermore, our tests with dimethylsulphoxide (DMSO) that enhances skin penetration never showed a generalized corticosteroid allergy, even though they did reveal some additional allergies (data on file). This makes it unlikely that all corticosteroids cross-react with each other.

The principal variants in the corticosteroid cyclopentanophenanthrene structure and substitutes are (Fig. 1): A-ring, double bond C_1–C_2; B-ring, substitutions at C_6, C_9, C_7 (fluorine, chlorine, and methyl groups); D-ring -16, 17-*cis*-diol with *cis*-ketal formation and -16-methyl substitute; side chain, esterification C_{17} and/or C_{21}.

It needs to be considered whether the parameters that can preferentially explain associated positive patch test results (APPR) between corticosteroids are structural variations in the A- and B-rings of the molecule (Hypothesis 1, Table 1) or in the D-ring or C_{20}, C_{21}-side chain (Hypothesis 2, Table 2). For each of the two hypotheses, we will determine on the basis of these structural data whether significantly more APPR occur within these groups or between the molecules of the various groups.

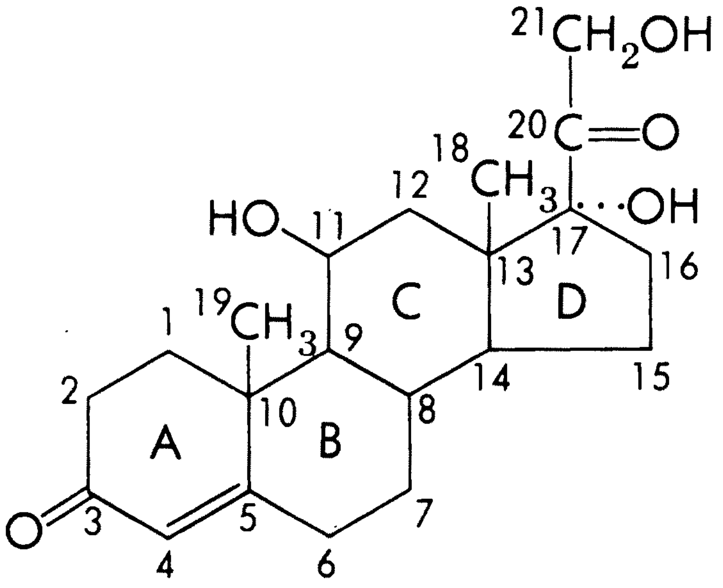

FIGURE 1. The structure and nomenclature of adrenocorticosteroids. The prototype is cortisol (hydrocortisone).

TABLE 1. Group definition under Hypothesis 1

Corticosteroid group	Double bond C_1–C_2 in the A-ring	X-substitute ($X = -F, -Cl, -CH_3$) in the B-ring	Type
A	–	–	hydrocortisone
B	–	C_9	halcinonide
C	+	–	prednisolone
D_1	+	C_6 (C_7)	fluocortolone
D_2	+	C_9	dexamethasone
D_3	+	C_6 and C_9	fluocinolone acetonide

A, cortisone, cortisone acetate, hydrocortisone, hydrocortisone acetate, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, tixocortol pivalate.

B, fludrocortisone, halcinonide.

C, budesonide, desonide, methylprednisolone acetate, prednisolone, prednisolone acetate, prednisone.

D_1, fluocortolone, fluocortolone caproate, fluocortolone pivalate, alclometasone dipropionate.

D_2, amcinonide, betamethasone, betamethasone sodium phosphate, betamethasone valerate, betamethasone dipropionate, clobetasol-17-propionate, clobetasone-17-butyrate, cloprednol, dexamethasone, dexamethasone sodium phosphate, fluprednidene acetate, triamcinolone acetonide, triamcinolone alcohol.

D_3, fluocinonide, fluocinolone acetonide.

Only the corticosteroids involved in the present study are listed.

METHODS

An analysis was conducted of associated positive patchtest results (APPR) for corticosteroids reported in the literature[6–30] and our own patch test series.[31] We chose a descriptive approach as it was considered inappropriate to apply a rigorous statistical method since some patients manifest several APPR unlike others and the data were not gathered randomly. The criteria for data selection were as follows:

Unambiguous test procedure

Tests were made with the corticosteroids diluted in ethanol or petrolatum or with the commercial preparations with exclusion of a contact allergy to all other components.

Unambiguous test results

At least one positive patchtest reaction (i.e. weak; non-vesicular reaction) (ICDRG coding system); doubtful reactions (?+) were not considered.

Homogeneity of the compiled data

In order to assure internal comparability of the test conditions the epicutaneous tests were made without any penetration enhancing agents such as DMSO and with reading times up to 120 h after application.

Test readings

At most five positive patch tests were done to avoid the 'excited skin syndrome'.[32] Unreliable

TABLE 2. Group definition under Hypothesis 2

Corticosteroid group	C_{16} substitution	Carbon chain at the ester of C_{17} and/or C_{21}	Type
A	–	⩽ acetate or thioesters	hydrocortisone tixocortol pivalate
B	$-OH \pm 16, 17$-cis ketalation	⩽ acetate	triamcinolone acetonide
C	$-CH_3$	–	betamethasone
D	–	> acetate	hydrocortisone-17-butyrate
	$-CH_3$ or $=CH_2$	⩾ acetate	clobetasone-17-butyrate

A: Cloprednol, cortisone, cortisone acetate, fludrocortisone, hydrocortisone, hydrocortisone acetate, methylprednisolone acetate, prednisolone, prednisolone acetate, prednisone, tixocortol pivalate.

B: Amcinonide, budesonide, desonide, fluocinolone acetonide, fluocinonide, halcinonide, triamcinolone acetonide, triamcinolone alcohol.

C: Betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone.

D: Alclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, clobetasol-17-propionate, clobetasone-17-butyrate, fluocortolone caproate, fluocortolone pivalate, fluprednidene acetate, hydrocortisone-17-butyrate, hydrocortisone-17-valerate.

Only the corticosteroids involved in the present study are listed.

data from even a small number of patients would blur the cross-reaction tendencies observed in the global population.

RESULTS

The results with respect to these two hypotheses (Tables 1 and 2) with correction for the size of the categories (internally in the groups versus externally between the groups) and possibly selective test behaviour of the authors, are presented in Figures 2 and 3. The two hypotheses are compared in Table 3. For both, we have calculated the ratio of the values of the intragroup and intergroup parameters.

After correction for the size of the two categories, 10 times more APPR (10:1) occurred between corticosteroids with an analogous substitution on the D-ring and C_{20}, C_{21}-carbon chain (Hypothesis 2, cf. Table 2). This ratio is only 1.5:1 for corticosteroids with similar substitutions on the A-and B-rings (Hypothesis 1, cf. Table 1). A correction for selective test behaviour (Parameter B in Table 3) seems primarily necessary for Hypothesis 2 (ratio 1·5:1). Even then (parameter A/B), the chance of APPR between corticosteroids with analogous substitution on the D-ring or the C_{20}, C_{21}-side chain is 6·6 times greater than when these substitutions belong to a different class. With regard to the substitutions on the A- or B-rings, this ratio is 1·4:1. These results suggest the following classification (cf. also Table 2):

Class A, Hydrocortisone type, corticosteroids without substitution on the corticosteroid D-ring or C_{20}, C_{21}-carbon chain, but including C_{17}- and/or C_{21}-short chain, (acetate) esters and the C_{21}-thioester, tixocortol pivalate.

Class B, Triamcinolone acetonide type, corticosteroids with a C_{16}, C_{17}-*cis*-ketal or -diol structure.

TABLE 3. Comparison of test results in terms of Hypotheses 1 and 2

	Hypothesis 1			Hypothesis 2		
Parameter	Intragroup	Intergroup	Ratio	Intragroup	Intergroup	Ratio
No. of reported APPR	60	68		98	30	
No. of possible APPR = size of category	208	353		138	423	
No. of SAP	998	1582		858	1722	
$\frac{\text{No. of reported APPR}}{\text{Size of category}} = A$	0·2885	0·1926	1·5:1	0·7101	0·0709	10:1
$\frac{\text{No. of SAP}}{\text{Size of category}} = B$	4·7981	4·4816	1·1:1	6·2174	4·0709	1·5:1
$\frac{\text{No. of reported APPR}}{\text{No. of SAP}} = \frac{A}{B}$	0·0601	0·0430	1·4:1	0·1142	0·0174	6·6:1

APPR: associated positive patch test results.
SAP: simultaneously applied patch tests.
Ratio: The intragroup parameters divided by the intergroup parameters.

Class C, Betamethasone type, corticosteroids with a C_{16}-methyl substitution.
Class D, Hydrocortisone- and clobetasone-17-butyrate type, corticosteroids with a long-chain ester at C_{17} and/or C_{21}, and with or without a C_{16}-methyl substitution.

DISCUSSION

Evaluation of the two hypotheses

A systematic analysis of the corticosteroids patch test results reported in the literature as well as those obtained from our own patch test data demonstrates that, in contrast with the randomly

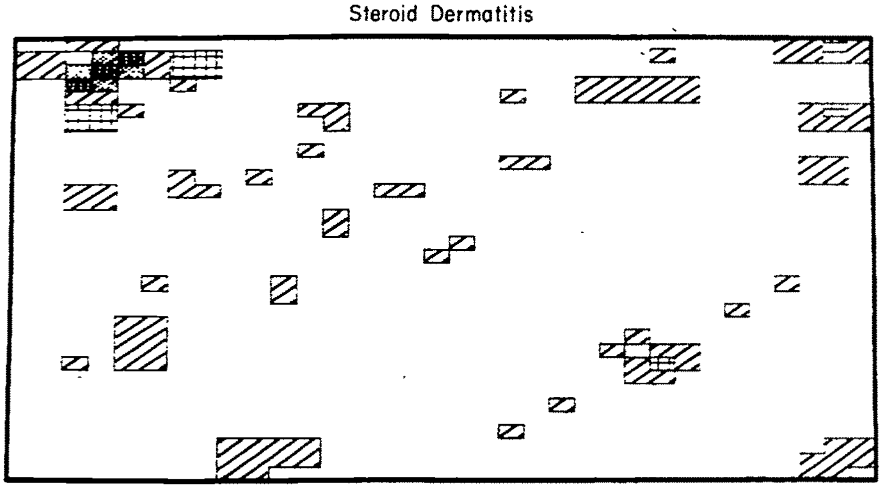

FIGURE 2. Associated positive patch test reactions with respect to hypothesis 1 (cf. Table 1). The corticosteroids from Groups A to D are listed in the same order along the abcis and ordinate.

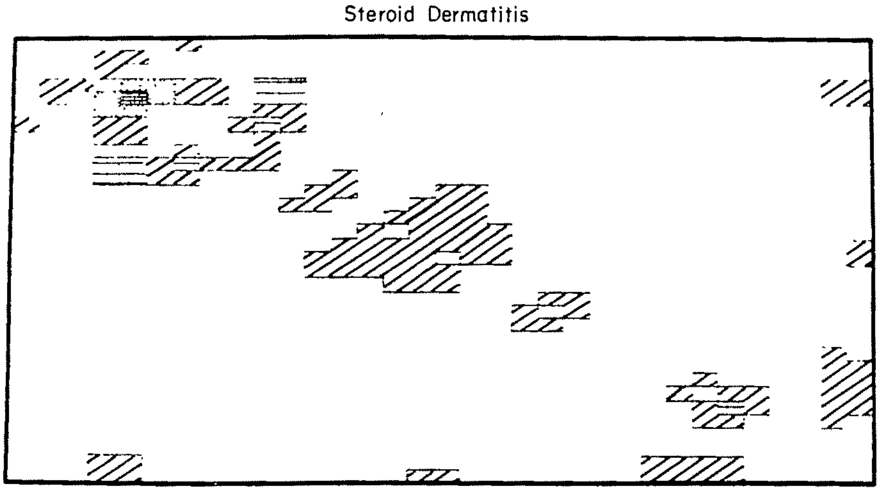

FIGURE 3. Associated positive patch test reactions with respect to hypothesis 2 (cf. Table 2). The corticosteroids from Groups A to D are listed in the same order along the abcis and ordinate.

distributed APPR (Fig. 2), the positive results (Fig. 3) are situated within well determined structural groups as suggested in Hypothesis 2. It is clear that analogous substitutes on the D-ring and the C_{20}, C_{21}-side chain of the corticosteroid molecule associate better with the observed APPR.

Whether the results of Hypothesis 1 (ratio of 1·4 to 1) are within the range of pure chance is less clear. We are of the opinion, however, that this can hardly be expected because substitutions at different locations of the molecule are not completely independent. Thus, the increase of 'general' corticosteroid activity by fluoride substitutions on C_6 or C_9 requires an enhanced dissociation of the gluco-and mineralocorticoid activity in order to reduce the side-effects of the latter, which, for example, is obtained by substitution at C_{16}. Further data are needed for a more accurate evaluation.

The success of the predictions of Hypothesis 2 is remarkable. The ratio of 6·6 to 1 can hardly be explained by concomitant sensitization because of the chemical relationship between the reacting corticosteroids. One would expect preferential prescription of products that are of a different nature (or at most, 'random prescription behaviour') in the clinical context of, e.g. a therapy-resistant eczema. The data strongly suggest a cross-allergy phenomenon underlying the observed associated positive patch test results.

The specificity of the associated positive patch test results

For the APPR that do not fit into Hypothesis 2, several explanations can be suggested: concomitant sensitization, skin metabolism, and an intrinsically limited specificity of cross-allergy. Most of the patients had used a large number of corticosteroid preparations on eczematous skin over a long period of time. Thus, it is not surprising that they often developed multiple sensitivities,[23] even to other corticosteroids. Corticosteroid esterase activity in the skin[33] could be responsible for the conversion of hydrocortisone-17-butyrate to hydrocortisone and fluocortolone caproate or pivalate to fluocortolone. However, this esterase activity in human skin is limited, which justifies the distinction of structural Group D from Groups A and C. Thioesters such as tixocortol pivalate are capable of undergoing rapid hydrolysis in various organs (Jouveinal Co., Product Information) whereby free tixocortol that is generated differs from hydrocortisone only by a thiol group instead of a hydroxyl group at C_{21}. For this reason, it is not surprising that tixocortol pivalate cross-reacts with corticosteroids in Group A. Furthermore, a sulphur atom differs from an oxygen atom in its nucleophilic reactivity and oxidability, which may be expressed in the ability of tixocortol pivalate to function as a contact allergy marker.[2]

A certain continuity in the transition between the various defined groups cannot be denied. Firstly there is the difficult position of the short-chain esters. We have drawn the line between Groups A and D somewhat arbitrarily at the acetates, which clearly react in several groups and thus could be responsible for a number of ill-fitting APPR. Some corticosteroids consist of combinations of different substitutes such as amcinonide, which is a 16, 17-*cis*-ketal-21-acetate.

From a more theoretical point of view, the chemical affinity in each interaction between molecules, and thus, also of an allergen and its target, has to be considered as the result of two elements, an intrinsic molecular affinity and a concentration effect *in situ*.[34] It would be interesting to determine the influence of a penetration enhancing agent such as dimethylsulphoxide (DMSO) on the specificity of the cross-allergy patterns. This problem is currently being investigated in our department. This is of interest to the clinician, since the damaged skin to which the corticosteroids are generally applied does constitute a DMSO-like situation. It is essential that the extent of the specificity of cross-reactions as observed in patch tests is relevant to the therapeutic situation. It is too soon for a definitive answer. However, we have noted that all of our patients displayed clinical improvement upon the application of a corticosteroid of a group to which no reaction was obtained in the classic patchtest procedure.

The problem of multiple positive reactions to corticosteroids observed in a minority of patients needs further investigations.

Sensitivity of the corticosteroid cross-reaction phenomenon

As the specificity of the phenomenon is relevant primarily to the therapeutic choice, the sensitivity of cross-reactions between corticosteroids of the same structural group is of importance for the determination of suitable allergy markers. The average number of APPR per simultaneously applied patch test (SAP), i.e. A/B (cf. Table 3) for two corticosteroids of the same group (intragroup) in Hypothesis 2 is quite small, namely 0·11 (roughly one out of nine). This is unimportant since we are not so much interested in an overall cross-reaction rate as in a reliable cross-allergy pattern for specific group markers. For example, in our patch-test series, the diagnostic sensitivity of tixocortol pivalate in patients with contact allergies to corticosteroids of Group A is so far 100%. We are now attempting to optimize the choice of appropriate allergy markers and test conditions for Groups B, C, and D, since we consider that the most reliable strategy for the detection of corticosteroids allergy at present, consists in patchtesting certain representatives of the four groups as indicated in our analysis.

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Antibodies to Ro/SSA detected by ELISA: correlation with clinical features in systemic scleroderma

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SUMMARY

Anti-Ro/SSA antibodies were determined by a newly developed enzyme-linked immunosorbent assay in serum specimens from 114 patients with systemic scleroderma in order to examine the relationship between Ro/SSA antibodies and clinical subsets of scleroderma. Sera of 42 patients (37%) were positive for Ro/SSA antibodies. Clinical investigations, including Schirmer's test and enzymatic profiles, demonstrated that 60% (16 of 27) of scleroderma patients with sicca syndrome and 63% (10 of 16) with polymyositis (PM) were Ro/SSA positive. In these patients there was a significant association between Ro/SSA antibodies and rheumatoid factor. HLA-antigens DR2, DR3 and B8 showed an increased frequency in anti-Ro/SSA positive patients.

Systemic scleroderma (SS) is a generalized disorder of the skin and other connective tissue containing organs and is characterized by fibrosis and vascular alterations of the skin, gastrointestinal tract, lungs heart and kidneys. It can present in different forms such as acroscleroderma, with limited cutaneous and extracutaneous involvement, and diffuse scleroderma with a more rapid progression.[1]

Recently, the finding of circulating autoantibodies in systemic scleroderma against nuclear and nucleolar antigens has attracted much attention. These immunological markers may be helpful in establishing a classification and for distinguishing certain subsets of the disease.[1,2] Antibodies have been demonstrated against components of the cell nucleus such as DNA-topoisomerase I,[3] U1-RNP[4] and kinetochore proteins[5] or against nucleolar particles including RNA-polymerase I, U3-RNP (fibrillarin) and the PM-Scl-antigen.[2] With the exception of U1-RNP antibodies that are more frequent in patients with lupus erythematosus or with mixed connective tissue disease,[4] these autoantibodies show a high degree of specificity for systemic scleroderma. A second antibody system directed against the cytoplasmic Ro/SSA antigen has been described in some patients with systemic scleroderma.[6,7] The correlation of these

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cytoplasmic antibodies with clinical features, has been mainly investigated in patients with lupus erythematosus.[7-9] The purpose of this study was to determine the prevalence of Ro/SSA antibodies in a large group of patients with different forms of systemic scleroderma and to investigate their association with certain clinical manifestations of systemic scleroderma.

METHODS

Patients

One hundred and fourteen patients (93 female and 21 male, mean age 56·4) were investigated. These patients were referred to the Scleroderma Clinic in the Department of Dermatology at the Ludwig-Maximillians University, Munich and all fulfilled the American Rheumatism Association (ARA) criteria for the diagnosis of systemic scleroderma.[10]

According to the classification by Barnett[11] 34 patients were diagnosed as having acrosclero-derma (type I), 77 patients had proximal ascending scleroderma (type II) and three patients had truncal scleroderma (type III). According to the recently proposed classification of LeRoy *et al.*[12] for scleroderma, type I (including CREST syndrome) and type II would in most cases correspond to 'limited cutaneous sclerosis', whereas type III corresponds to "diffuse cutaneous sclerosis". Esophageal motility was assessed by conventional radiography, manometric measurements and by scintigraphy procedures. Lung involvement was determined using routine X-ray procedures, functional tests, scintigraphy and bronchoalveolar lavage. Echocardiography was used to detect cardiac involvement. The criteria for renal disease include persistent proteinuria (500 mg/24 h) or hypertension (140/90 mmHg) or azotaemia (blood urea nitrogen >5 mg/100 ml). Arthralgia and signs of inflammation and synovial effusions were criteria for joint involvement. The sicca syndrome was diagnosed in patients with xeropothalmia confirmed by a positive Schirmer test and/or persistent xerostomia with parotid gland swelling. Polymyositis was diagnosed in patients with weakness and tenderness or wasting of the proximal muscles accompanied by elevated serum creatine levels.

Purification of the Ro/SSA antigen

Ro/SSA antigen was extracted from fresh human spleen tissue obtained within 24 h post mortem according to the method described by Clark *et al.*[13] The Ro/SSA fraction was further purified by affinity chromatography as outlined in detail elsewhere.[14] The purified antigen was characterized by size separation in 10% polyacrylamide gel electrophoresis (PAGE) using sodium dodecyl sulphate (SDS) according to the method described by Laemmly.[15] Protein staining showed a peptide of 58 kDa and an additional protein of 14 kDa molecular weight as major components of the isolated Ro/SS antigen (Fig. 1, Lane A).

The Ro/SSA antigenic activity was assessed by Western blot analysis.[16] Figure 1 shows that only the 58 kDa protein was reactive with standard sera (Lane B); no reaction was noted with normal human control serum (Lane C).

ELISA

Ro/SSA antibodies were detected by an enzyme-immunosorbent assay (ELISA) following a previously described method,[14,17] though with some modifications. The purified Ro/SSA antigen fraction was incubated with RNase (v/wt: 5 ml/3mg) and 50 μg 0·5 M $MgCl_2$ (1 h, 37°C). RNase digested antigen diluted in saline citrate buffer, pH 9·5 (10–20 μg/100 μl) was coated overnight onto the wells of Immulon plates (Dynatech Corp., Baton Rouge, LA, U.S.A.). After

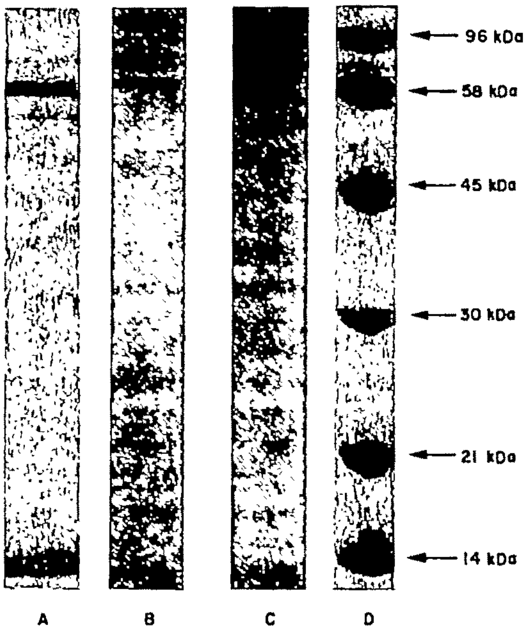

FIGURE 1. Identification of Ro/SSA antigen. A, SDS polyacrylamide gel electrophoresis (10%), staining pattern of affinity purified Ro/SSA antigen; B, Western blot of Ro/SSA preparation using Ro/SSA standard reference serum, a polypeptide of 58 kD molecular weight was reactive with human anti-Ro/SSA antibodies; C, Western blot analysis using normal human sera; D, Marker proteins (LMW-Bio Rad).

blocking remaining binding sites with 0·1% bovine serum albumin, 100 μl of serum diluted in PBS-Tween (1:100–1:200) were added to the plate and incubated (1 h, 20°C). After washing, bound antibodies were stained by goat anti-human IgG conjugated to peroxidase (1:1000, 1 h, 20°C) (Sigma Chemical Co., St. Louis, MO, U.S.A.). In the final step the plate was incubated with an orthophenylendiamine substrate solution and subsequent chromophore development was measured at 405 nm by an automatic multiscan plate reader (Flow).

One Ro/SSA positive standard serum and four negative control sera were used as standards. Reactions were considered positive if the absorbance was higher than three standard deviations above the mean value of the four negative control sera.

Standard immunological techniques

Antinuclear antibodies were detected by an immunofluorescence technique using HEp-2-cells (Kallestad labs Inc, Chaska, MN, U.S.A.) as antigen substrate, and the rheumatoid factor (RF) was determined by a latex fixation test. Precipitating antibodies against La(SSB) and Scl-70 were detected by gel double-diffusion using rabbit thymus extract as antigen sources.[3,13] Creatinephosphokinase were measured following standarized methods.

HLA-A, -B, -C and -DR specificities were determined in some patients using standardized microcytoxicity assays (Prof. Albert, National Reference Laboratory for Tissue Typing Childrens Hospital, Universität München).

Clinical data were analyzed using χ^2-test for statistical evaluation. *P* values less than 0·05 were considered significant.

TABLE 1. Clinical type and organ involvement in scleroderma patients with or without Ro/SSA antibodies

	Ro/SSA+	Ro/SSA−	χ^2-test
Type I	11 of 42 (26%)	23 of 72 (32%)	N.S.
Type II	29 of 42 (69%)	48 of 72 (67%)	N.S.
Type III	2 of 42 (5%)	1 of 72 (1%)	N.S.
Oesphagus	34 of 42 (81%)	42 of 72 (58%)	N.S.
Joint	22 of 42 (52%)	34 of 72 (47%)	N.S.
Lung	22 of 42 (52%)	24 of 72 (33%)	N.S.
Heart	9 of 42 (21%)	11 of 72 (15%)	N.S.
Kidney	16 of 42 (38%)	17 of 72 (24%)	N.S.
Sicca syndrome	16 of 42 (38%)	11 of 72 (15%)	$P \leqslant 0\cdot01$
Polymyositis	10 of 42 (24%)	6 of 72 (8%)	$P \leqslant 0\cdot01$

χ^2-test (0·01, 1 FG) = 7·60.

RESULTS

Ro/SSA antibodies in patients with systemic sclerosis

The sera of 114 patients were evaluated by the newly developed ELISA and Ro/SSA antibodies were detected in 42 of 114 (37%) scleroderma patients, whereas 72 (63%) were Ro/SSA antibody negative.

The presence or absence of antibodies to Ro/SSA in the three clinical subtypes are shown in Table 1 and in these groups no statistically significant difference was found as regards antibody prevalence. There were no difference in major organ involvement, i.e. oesophagus, lungs, heart, kidneys and joints, between the Ro/SSA positive and negative patients. However, the sicca syndrome and polymyositis were more frequent in patients with the Ro/SSA antibody than in the antibody negative group. With these manifestations the differences were statistically significant ($P \leqslant 0\cdot01$). Of the 27 patients with sicca syndrome and systemic scleroderma (mostly

TABLE 2. Immunological findings in scleroderma pateints with or without Ro/SSA antibodies

	Ro/SSA+	Ro/SSA−	χ^2-test
ANA	42 of 42 (100%)	62 of 72 (86%)	$P \leqslant 0\cdot025$
AcA	5 of 42 (12%)	14 of 72 (19%)	N.S.
Anti-Scl-70	11 of 42 (26%)	12 of 72 (17%)	N.S.
Anti-La/SSB	2 of 42 (5%)	1 of 72 (1%)	N.S.
RF	27 of 42 (64%)	18 of 72 (25%)	$P \leqslant 0\cdot01$

χ^2-test (0·025; 0·01, 1 FG) = 6·39; 10·02.
ANA, antinuclear antibodies.
AcA, anticentromere antibodies.
RF, rheumatoid factor.

TABLE 3. HLA-DR2, -DR3, -B8 specificities in 39 scleroderma patients with or without Ro/SSA antibodies.

	Ro/SSA +	Ro/SSA −	χ^2-test
HLA-DR2	12 of 27 (44%)	4 of 12 (33%)	N.S.
HLA-DR3	8 of 27 (30%)	1 of 12 (8%)	N.S.
HLA-B8	1 of 12 (17%)	2 of 12 (17%)	N.S.

type II), 16 (60%) had Ro/SSA antibodies. Of the 16 patients with polymyositis and scleroderma, 10 (63%) were positive for these antibodies.

The results of other serological tests performed in patients with and without Ro/SSA antibodies are listed in Table 2. The association of Ro/SSA antibodies with antinuclear antibodies detected by indirect immunofluorescence using HEp-2-cells as substrate was significant ($P \leqslant 0{\cdot}025$). Other serum autoantibodies such as anti-Scl-70 and anti-centromere antibodies were less frequently associated with Ro/SSA antibodies and only 5% of patients with Ro/SSA antibodies were shown to have antibodies against the La/SSB antigen. Rheumatoid factor (RF) was significantly more frequent ($P \leqslant 0{\cdot}01$) in the Ro/SSA antibody positive than in the antibody negative group.

HLA-typing (Table 3) was performed only in a limited group of patients and did not reveal any statistical difference in patients with or without Ro/SSA antibody. However, eight out of 27 (30%) scleroderma patients with Ro/SSA antibodies had HLA-DR3 compared with only one out of 12 (8%) patients without Ro/SSA antibodies. HLA-B8 was found in eight out of 27 (30%) Ro/SSA-positive patients but in only two out of 12 (17%) patients without Ro/SSA antibodies.

DISCUSSION

Antibodies directed against the Ro antigen were first described in some patients with systemic lupus erythematosus,[13] while antibodies to the SSA antigen were initially detected in patients with Sjögren's syndrome.[18] It was later discovered that the Ro and SSA antigens were immunologically identical.[19] More recently it was shown that the binding site of Ro/SSA antibodies resides in the protein component of small cytoplasmic ribonucleoprotein particles (scRNPs), and has a molecular weight of approximately 60 kDa.[20] The RNA component of the Ro/SSA scRNP is composed of four human RNAs of small size, designated hYl, hY3, hY4 and hY5.[21]

Ro/SSA antibodies can be detected by immunodiffusion technique, by counterimmunoelectrophoresis or by ELISA.[14,17] For this study we have modified the sensitive ELISA technique using affinity purified Ro/SSA antigen. Our Ro/SSA antigen preparation was shown to have a molecular weight of 58 kDa, which corresponds to previously described biochemical data.[14,20,22] By this ELISA technique we have found Ro/SSA antibodies in 37% of scleroderma patients and could demonstrate an association between the antibodies and clinical features of sicca syndrome and polymyositis in these patients.

Manifestations of sicca syndrome in patients with systemic scleroderma have been reported by Alarcon-Segovia[23] in 84% and by Cipoletti[24] in 34% of cases. Histological changes of Sjögren's syndrome diagnosed by minor salivary gland biopsies were, however, only present in

52%[23] and in 17%,[24] respectively, of these patients. Ro/SSA or La/SSB antibodies were not investigated in these studies. Similarly, clinical signs of sicca syndrome were found in 28% and biopsy proven Sjögren's syndrome in 29% of scleroderma patients.[25] These workers noted that 53% of the patients with proven Sjögren's syndrome and scleroderma had anti-Ro/SSA and/or La/SSB antibodies.[25] Our study shows a similar correlation between Ro/SSA antibodies and sicca syndrome as determined by the presence of xerophthalmia, xerostomia and pathological Schirmer test. About 60% of the scleroderma patients with sicca syndrome were positive for Ro/SSA antibodies.

La/SSB antibodies were only found in three of the patients with sicca syndrome, indicating that this type of antibody is less frequently associated with Ro/SSA in scleroderma than in certain subtypes of systemic lupus erythematosus or in primary Sjögren's syndrome.[26]

Ro/SSA antibodies were reported in two studies to be present in between 8 and 38% of patients with primary polymyositis.[27,28] Clements found an overlap of polymositis and systemic sclerosis in 17% of their patients.[29]A relationship between the occurrence of Ro/SSA antibodies and polymyositis in patients with systemic sclerosis has not previously been described. Our data demonstrate that the prevalence of Ro/SSA antibodies is even higher in patients with scleroderma and secondary polymyositis. Also in our study cytoplasmic Ro/SSA antibodies were more frequently associated with polymyositis–scleroderma overlap syndrome than were anti-PM-Scl antibodies. These antibodies which are directed against a set of nucleolar proteins[2] were found in only four of 16 patients with this overlap syndrome.

The occurrence of hypergammaglobulinaemia, cryoglobulinaemia, hypocomplementaemia and rheumatoid factor in patients with Ro/SSA antibodies has been noted by several authors.[7,30,31] In our study we found a significant association between rheumatoid factor and Ro/SSA antibodies in patients with scleroderma ($P \leqslant 0{\cdot}01$), confirming similar results in lupus erythematosus.[26] All our scleroderma patients with Ro/SSA antibodies also had a positive ANA ($P \leqslant 0{\cdot}025$) as detected by indirect immunofluorescence on HEp-2-cells. In contrast to lupus erythematosus[32] we have not found ANA-negative scleroderma patients with Ro/SSA antibodies.

In patients with primary Sjögren's syndrome and in some patients with subacute cutaneous lupus erythamatosus, Ro/SSA antibodies were found to be highly associated with HLA-DR2, -DR3 and -B8.[9,30,33] In polymyositis an association with HLA-B8 was observed.[27] Our study demonstrated an increased frequency of HLA-DR2, -DR3, and -B8 in scleroderma patients with the sicca syndrome and/or polymyositis and Ro/SSA antibodies. These findings suggest that in scleroderma there is a similar correlation of HLA specificities with Ro/SSA antibodies to that reported in lupus erythematosus.[34]

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The effects of vitamin E deficiency on rat skin

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SUMMARY

The effect of vitamin E deficiency on the levels of lipid peroxide and solubility of collagen was investigated in Wistar rats that were fed a vitamin E deficient diet for 3 and 6 months. The lipid peroxide content in the skin and subcutaneous tissue was markedly increased as compared with that of control rats on a normal diet. The lipid peroxide content in the tissues of rats deficient in vitamin E for 3 months, was greater than that found in those on the diet for 6 months.

The effect of UV irradiation on the lipid peroxide content in the skin of control rats was not significant, but there was a marked difference in lipid peroxide content in those animals on a deficient diet. The amount of insoluble collagen in the skin of rats on a vitamin E deficient diet for 6 months was increased and by more than in those animals on the diet for 3 months. The amount of soluble collagen in the rats deficient in vitamin E for 6 months was markedly reduced when compared with those on the diet for 3 months. These results suggest that vitamin E deficiency promotes the peroxidation of lipids and accelerates the cross-linking of collagen in the skin.

Vitamin E was first recognized as affecting fertility in rats.[1] It is a strong antioxidant and prevents cell damage caused by lipid peroxidation, and as an antioxidant, it acts as a free radical scavenger[2] protecting cells from oxidative damage. Thus, vitamin E must be considered as an important lipid antioxidant and is possibly involved in the inhibition of lipid peroxidation in the ageing process.[3] Very little information has been available on the effect of vitamin E on the skin though it has been demonstrated in rat skin that vitamin E deficiency causes an increase in soluble collagen,[4] but Blackett *et al.* found that in mice, the addition of vitamin E to the diet had no effect on the collagen content of the skin.[5] We have carried out studies on the production of lipid peroxide and the effect on skin collagen in rats fed on a diet deficient in vitamin E and compared with animals on a normal diet.

METHODS

Animals and diets

Two groups of five male weanling Wistar rats were raised on diets shown in Table 1. Both diets were in the form of powder and the vitamin E sufficient diet contained 23 mg dl-α-tocopherol acetate per 100 g. The stripped corn oil was purchased from Eisai Co. Ltd, (Tokyo), and the animals were maintained on these diets for up to 6 months.

TABLE 1. Component of vitamin E deficient diet and vitamin sufficient diet

	Vitamin E deficient diet	Vitamin E sufficient diet (control diet)
Cornstarch	36%	36
Vitamin free casein	25	25
α-starch	10	10
Cellulose powder	8	8
Minerals	6	6
Vitamins	2	2
Granulated sugar	5	5
Stripped corn oil	8	8
dl-α-tocopheryl acetate	—	23 mg/100 g

Haemolysis test

The determination of haemolysis with dialuric acid was carried out as described by Ikeda and Sugiyama.[6] Dialuric acid was purchased from Tokyo Kasei Kogyo Co. Ltd, (Tokyo) and its solution was prepared immediately before use.

Determination of vitamin E content

The vitamin E (α-tocopherol) content in the tissues was determined by the modified method of Katsui as follows.[7] After 1 ml of serum was shaken with 1 ml distilled water, the solution was mixed with 2 ml ethanol and then 10 ml hexane was added. The mixture was vigorously shaken with 10 ml *n*-hexane. After centrifugation (1,000 *g* for 5 min), *n*-hexane extracted fraction (8 ml) was evaporated and then an accurate volume of methanol was added to the residue. The other tissues were homogenized with 6-fold distilled water and then methanol was added. The mixture was vigorously shaken with *n*-hexane. After centrifugation (2,000 *g* for 10 min), the *n*-hexane extracted fraction was evaporated and an accurate volume of methanol was added to the residue. The methanol solution was analyzed using high pressure liquid chromatography (HPLC). Vitamin E was detected with UV detector at 280 nm. The analysis was carried out by HPLC with 6000 pump and the U6K injector of Waters Assoc. (Tokyo). The column used was Radial-Pak A (silica ODC) of Japan Waters Limited.

Determination of lipid peroxide

The lipid peroxide content of skin and subcutaneous tissue was determined by the TBA method as described by Yagi *et al.*[8] Skin and subcutaneous tissue were washed after removal in ice cold buffer, then minced and homogenized in the cold with 0·15 M KCl with 10 mM Tris buffer. The homogenate was then centrifuged at 2,000 *g* for 10 min and to 2 ml of supernatant, 2 ml of 7% sodium dodecyl sulphate, 1 ml of 0·67% thiobarbituric acid, and 2 ml of 50% acetic acid were added. The mixture was heated at 95°C for 1 h, and the red chromophore, a reaction product of lipid peroxide with thiobarbituric acid, was extracted using a mixture of butanol and pyridine. Following centrifugation, the red chromophore in the butanol–pyridine fraction phase was then measured at 532·5 nm.

UV irradiation

Rats raised on a vitamin E deficient and on control diets for 3 months and 6 months were irradiated with a bank of six 40w Toshiba fluorescent lamps for 2·5 h. The irradiated backs of the rats were 30 cm from the light source. At this distance the irradiance, as measured by a UV radiometer (Eisai, Co Ltd.), was 0·578 mW/cm^2. After exposure, the rats in each group were killed and content of lipid peroxide in the skin and subcutaneous tissue determined.

Determination of collagen

After removal of the subcutaneous tissue, the skin was minced and homogenized at 4°C with 20 mM Tris-HCl and 0·45 M NaCl at pH 7·4. The soluble collagen was removed by incubating the homogenate for 24 h at 4°C and then it was centrifuged at 12,500 *g* at 4°C for 30 min. The insoluble collagen was isolated by incubating the precipitate for 30 min at 90°C with 5% trichroloacetic acid and extracted in the supernatant after centrifuging at 2,000 *g* for 15 min. The soluble and insoluble collagen extracted from the tissue were assayed for hydroxyproline[9,10] and which reflected the collagen content.

RESULTS

Haemolysis

Haemolysis started to increase within 5 days after starting on the vitamin E deficient diet and reached up to 80% haemolysis after 10 days and 100% haemolysis within a month. No haemolysis was observed in the control rats during this period. A small amount of vitamin E was still detectable in the serum of the animal even after a month on the vitamin E deficient diet.

Body weight

There were no significant difference in the growth of the animals in both groups.

Content of vitamin E

The vitamin E (α-tocopherol) contents in the skin and the other tissues are shown in Table 2. In the control group of rats, the vitamin E content ranged from 0·4 to 10 $\mu g/g$ depending on the tissue. In the vitamin E deficient rats, a small amount of vitamin E was detectable in the serum and liver, but in the skin and subcutaneous tissue none was detected. In rats fed on the diet deficient in vitamin E for 6 months, no vitamin E was detected in their tissues.

TABLE 2. Content of vitamin E (α-tocoperol) in the various tissues

	3 month		6 month	
	Control	Vitamin E deficient	Control	Vitamin E deficient
Serum ($\mu g/ml$)	7·72+0·55	0·20+0·02	1·26+0·08	N.D.
Liver ($\mu g/g$)	10·68+2·07	0·22+0·01	2·49+1·14	N.D.
Skin ($\mu g/g$)	0·39+0·02	N.D.	0·55+0·04	N.D.
Subcutaneous tissue ($\mu g/g$)	0·98+0·05	N.D.	0·48+0·04	N.D.

The values are expressed as $\mu g/g$ tissue; mean+SD of five rats determinations. N.D., not detected.

TABLE 3. Content of lipid peroxide in the skin and subcutaneous tissue

Tissue		Non UV-irradiated		UV-irradiated skin	
		3 month	6 month	3 month	6 month
Skin	Control	1·97+0·05	2·15+0·06	2·30+0·14	2·84+0·17
	Vitamin E deficient	3·10*+0·05	3·19*†+0·07	3·85+0·12	4·05+0·16
Subcutaneous tissue	Control	0·80+0·13	1·00+0·03	0·77+0·05	1·07+0·07
	Vitamin E deficient	1·68+0·21	2·25*†+0·83	2·57*+0·28	3·47*†+0·17

The values are expressed as nmol/mg protein; mean + SD of five rats determinations.
* < 0·001 in comparison to each normal group.
† < 0·05 in comparison to 3 and 6 months with vitamin E deficient rats.

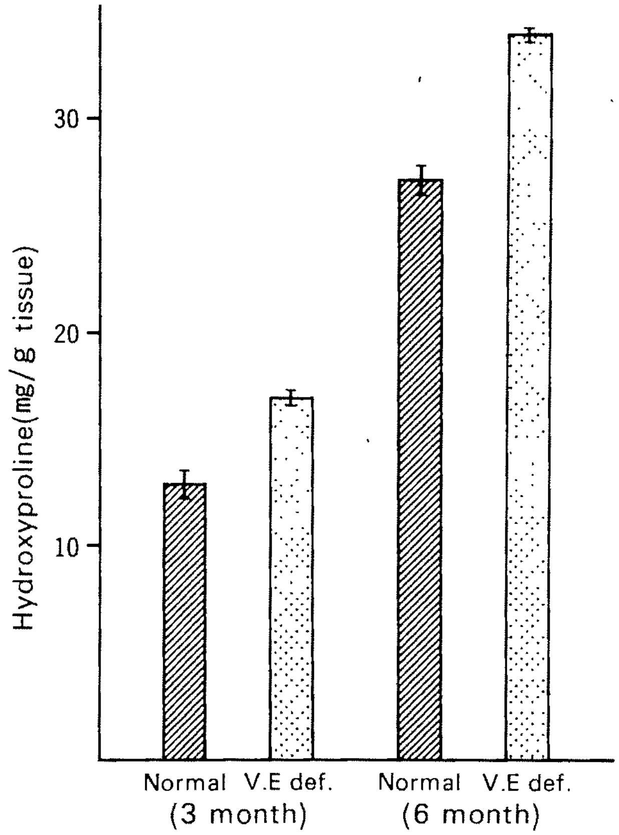

FIGURE 1. Content of insoluble collagen in the skin. ▨, rats fed with vitamin E sufficient diet; ░, rats fed with vitamin E deficient diet.

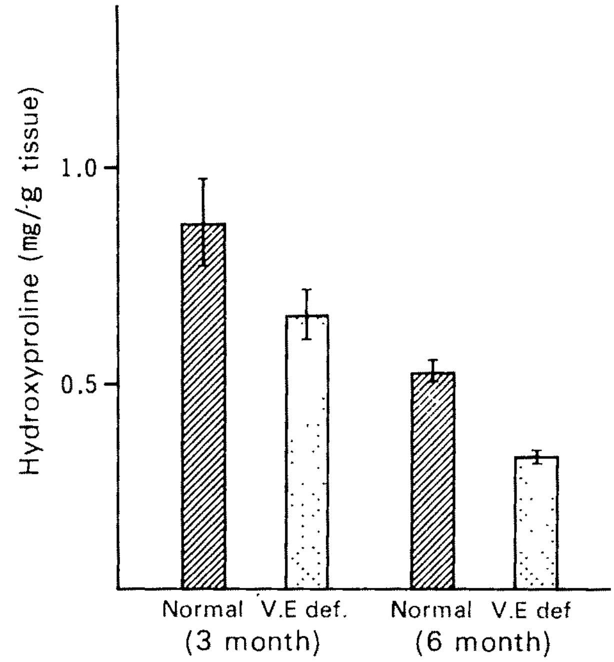

FIGURE 2. Content of soluble collagen in the skin. ▨, rats fed with vitamin E sufficient diet; ☐, rats fed with vitamin E deficient diet.

Lipid peroxide

The levels of lipid peroxide in the skin and subcutaneous tissue of vitamin E deficient rats are shown in Table 3. The vitamin E deficient rats showed high levels of lipid peroxide in both the skin and subcutaneous tissue when compared with that of controls. These differences were statistically significant ($P < 0{\cdot}001$). The difference in the lipid peroxide contents in the tissues between 3 and 6 months was greater ($P < 0{\cdot}05$) in the vitamin E deficient rats than the control animals.

The effect of UV irradiation was studied and in the controls there was no effect, but in vitamin E deficient rats the lipid peroxide contents of the skin after UV irradiation showed a significant increase. There was even more of an increase in these animals on vitamin E deficient diet for 6 months.

Collagen

Rats fed with the vitamin E deficient diet for 3 months showed a marked increase in the amount of insoluble collagen (Fig. 1). Those rats on a deficient diet for 6 months showed a greater increase in insoluble collagen as compared with the controls. Rats fed on a vitamin E deficient diet for 3 and 6 months showed a marked decrease in the amount of soluble collagen (Fig. 2).

DISCUSSION

Although a number of biochemical abnormalities were found in rats fed on a vitamin E deficient diet, no significant difference was found in the weight of the internal organs compared to controls. An increase in haemolysis was observed after only 10 days on a vitamin E deficient diet. This suggests that even a slight lack of vitamin E makes the erythrocyte membrane liable to oxidative damage. A decrease in the vitamin E content of the skin and subcutaneous tissue occurred within 3 months and resulted in increased lipid peroxidation. This suggests its important role as an antioxidant and it is probably lost quicker in the skin than in the other tissues.

Currently there is much interest in the ageing of skin and the effect of UV light in this process. In vitamin E deficient rats exposed to UV light, there was a marked increase in the level of lipid peroxide in both the skin and subcutaneous tissue as compared with control rats similarly treated. Lipid peroxides are oxidation substances of unsaturated fatty acids[11] and are produced by a number of factors, particularly UV light. A synthetic antioxidant was found to improve vitamin E deficiency[12] and this suggests that the physiological role of vitamin E is closely related to its antioxidant activity.[13] The amount of lipid peroxides in the serum of patients with facial hypermelanosis,[14] and atopic dermatitis was reported to be higher than in healthy subjects and there may be some relationship between lipid peroxides and skin diseases. The application of synthetic lipid peroxides on guinea-pig skin produced histological changes with thickening and oedema of the epidermis.[15] Our data suggest that vitamin E inhibits the production of peroxides and protects the skin from damage. Rats raised on a vitamin E deficient diet were found to have a greater percentage of soluble collagen in their skin.[4] It was suggested that there was a defect in the formation of intermolecular and intramolecular cross-linkages in the collagen. However, our studies have shown that, in rats on a vitamin E deficient diet, the soluble collagen is markedly decreased when compared with control animals. The difference in these results may be due to a different extraction method of soluble collagen. It is generally observed that with age the amount of soluble collagen becomes markedly decreased and insoluble collagen increased.[16] We suggest that vitamin E in the skin reduces the increase in the amount of insoluble collagen by inhibiting the formation of peroxides. The changes in the skin produced by a lack of vitamin E in the rat resemble those seen in ageing, and vitamin E may have a part to play in preventing ageing of the skin.

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Erythromycin resistant propionibacteria in antibiotic treated acne patients: association with therapeutic failure

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SUMMARY

Erythromycin resistant (Em^R) propionibacteria were isolated from the skin surface of 51% of patients treated with oral erythromycin and 42% of patients treated with topical clindamycin compared with 3% of untreated control subjects ($P < 0{\cdot}001$). Amongst the topical clindamycin-treated patients, there was a higher incidence of Em^R propionibacterial carriage in those patients who had previously been treated with oral erythromycin (64%) than in patients with no known previous exposure to erythromycin (20%; $0{\cdot}01 > P > 0{\cdot}001$). Patients responding to oral erythromycin treatment carried Em^R propionibacteria less frequently (24%) than patients who were not responding or who had relapsed (70%; $P < 0{\cdot}001$). These observations suggest that the use of oral erythromycin and/or topical clindamycin encourages the development of resistant propionibacteria and that the emergence of resistant strains is associated with therapeutic failure in erythromycin-treated patients. In total 63 resistant isolates were obtained from 52 subjects. There were 42 strains of *Propionibacterium acnes*, 16 strains of *Propionibacterium granulosum* and five strains of *Propionibacterium avidum*. The majority of isolates were inducibly or constitutively resistant to macrolide (e.g. erythromycin), lincosamide (e.g. clindamycin) and streptogramin B type antibiotics. Therefore, the isolates are phenotypically indistinguishable from the majority of Em^R bacteria in which resistance is due to methylation of 23S ribosomal RNA.

Oral erythromycin is not extensively used in acne therapy but is a second line drug in patients who fail to respond to one of the tetracyclines. Topical erythromycin is useful in the management of mild acne and is preferred by patients who cannot tolerate the more aggressive forms of topical therapy such as benzoyl peroxide. Erythromycin, like the other antibiotics used in the treatment of acne vulgaris, is thought to work by inhibiting the growth of, or exocellular product formation by, cutaneous propionibacteria, the organisms implicated in the pathogenesis of the disease.

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Erythromycin resistant isolates of cutaneous propionibacteria have now been detected in the U.S.A.,[1–4] the U.K.,[5,6] France (P. Courvalin, personal communication) and Japan.[7] Frequently the isolates are also resistant to clindamycin, a structurally unrelated antibiotic. In most bacterial genera, co-resistance to erythromycin (a macrolide) and clindamycin (a lincosamide) is conferred by target site modification due to the action of a 23S ribosomal methylase which may be coded for by any one of a series of *erm* (erythromycin ribosomal methylase) genes.[8–10] These genes are located on plasmids or transposons and are thus able to spread easily between different strains and species of bacteria. In recent years *erm* genes have been identified in most genera of bacteria in which they have been sought. Methylase genes also confer resistance to a third group of structurally unrelated antibiotics, type B streptogramins. Erythromycin resistance of this type, which can be inducible or constitutive, is, therefore, referred to as MLS_B resistance. The phenotypes of Em^R propionibacteria have recently been investigated.[11] The majority of strains demonstrate inducible or constitutive resistance to macrolide, lincosamide and streptogramin B antibiotics, which is consistent with the expression of methylase genes, although the determinants have not yet been isolated and characterized.

Two studies of non-responding acne patients have found an association between the carriage of Em^R propionibacteria and lack of response to oral erythromycin therapy.[4,6] However, in both studies, the incidence of resistant strains in responding patients was not determined. In the U.S.A. combined erythromycin and clindamycin resistant (Em^RLm^R) propionibacteria have also been isolated from patients treated with topical erythromycin or clindamycin.[1,3] Therefore, the aims of this study were to compare the incidence of Em^R and Em^RLm^R propionibacteria in treated and untreated subjects and to determine whether the development of resistance was associated with the clinical use of erythromycin or clindamycin or both. A third objective was to compare the incidence of resistant isolates in responding and non-responding acne patients, in order to confirm or refute the association of propionibacterial antibiotic resistance with therapeutic failure.

METHODS

Subjects

Fifty-one patients on oral erythromycin (500 mg b.d.) and 53 patients on topical clindamycin (1% w/v) were included in the study. At the time of sampling, all had been on therapy for a minimum of 4 months. Of the erythromycin treated patients, most of whom were also using benomyl peroxide topically, 21 were improving, 12 showed no overall change in acne grade and 18 were worsening or had relapsed. Thirty (57%) of the clindamycin treated patients were concomitantly receiving oral therapy with minocycline (10), doxycycline (eight), tetracycline (one), erythromycin (three) or trimethoprim (eight). Therefore, their degree of response to clindamycin could not be evaluated. In addition 28 (53%) of the clindamycin treated patients were known to have previously been treated with oral erythromycin. One hundred untreated control subjects, with no history of erythromycin or clindamycin therapy, were also studied.

Microbiology

Sample collection. Propionibacteria were obtained from the skin surface (face or back) by a detergent scrub technique.[12] Viable counts were determined by plating out serial ten-fold dilutions of the wash fluid onto Brain Heart Infusion agar (Difco Laboratories, East Molesey, Surrey, U.K.) containing 3% (w/v) additional glucose (BHIg) and 2 μg/ml furazolidone (BHIgF, Sigma Chemical Co., Poole, Dorset, U.K.) to inhibit the growth of staphylococci. Incubation was carried out for 7 days at 37°C in an anaerobic cabinet.

Detection of antibiotic resistance. Antibiotic resistant propionibacteria were enumerated directly from the skin by plating out undiluted wash fluid and a 10^{-2} dilution onto BHIgF containing 0·5 μg/ml erythromycin (Sigma) or 0·5 μg/ml clindamycin (a gift from Upjohn Ltd., Crawley, West Sussex, U.K.). The concentrations used represent five times the minimum inhibitory concentration (MIC) for 90% of isolates, and have been shown in a previous study to differentiate between sensitive and resistant isolates.[6] Incubation conditions were as above.

Bacterial identification. Propionibacteria were identified by their colony appearance and anaerobic growth and were speciated according to the method of Marples and McGinley.[13]

Determination of antibiotic susceptibilities. Minimum inhibitory concentrations of erythromycin, clindamycin or virginiamycin S were determined by agar dilution on BHIg. Inocula were prepared by dilution of a 48 h BHIg broth culture to give a standard inoculum of 10^4 colony forming units per spot delivered by a multipoint inoculator. Plates were incubated as above but for 72 h only. The MIC of each antibiotic for each organism was recorded as the lowest concentration yielding no growth or a barely visible haze as determined with the unaided eye.

Statistical analyses

These were carried out using a G-test with Williams' correction, where appropriate.

RESULTS

Erythromycin resistant propionibacteria were isolated from the skin surface of 47% of erythromycin or clindamycin-treated patients compared with 3% of untreated controls ($P<0{\cdot}001$). There was no significant difference in the incidence of Em^{R} propionibacterial carriage between erythromycn-treated (51%) and clindamycin-treated (42%) patients. Patients responding to oral erythromycin treatment carried Em^{R} propionibacteria less frequently than patients who were not responding or who had relapsed ($P<0{\cdot}001$, Table 1). Amongst the patients being treated with topical clindamycin, there was a higher incidence of Em^{R}

TABLE 1. Incidence of erythromycin resistant propionibacteria in various treatment groups

Therapy	Patients	Number (%) with resistant propionibacteria	Significance*	
			Within group	Versus control
Oral erythromycin	Responders (21)	5 (24)	$P<0{\cdot}001$	$0{\cdot}01>P>0{\cdot}001$
	Non-responders (30)	21 (70)		$P<0{\cdot}001$
Topical clindamycin	Previous oral erythromycin (28)	18 (64)	$0{\cdot}01>P>0{\cdot}001$	$P<0{\cdot}001$
	No known previous oral erythromycin (25)	5 (20)		$0{\cdot}01>P>0{\cdot}001$
None	Untreated (100)	3 (3)		

* G-test with Williams' correction.

TABLE 2. Minimum inhibitory concentrations of erythromycin, clindamycin and virginiamycin S for resistant strains of propionibacteria

Treatment group (number of strains)	Antibiotic	Number of strains inhibited at an MIC (μg/ml) of:*											
		<0·25	0·5	1·0	2	4	8	16	32	64	128	256	≥512
Untreated (3)	Erythromycin												3
	Clindamycin				3								
	Virginiamycin S							1		2			
Erythromycin treated (35)	Erythromycin			9	1							4	21
	Clindamycin	9	4	2	2		3	3	7	2		2	1
	Virginiamycin S					1		5	11	12	4	1	1
Clindamycin treated (25)	Erythromycin			1	3					1	1		19
	Clindamycin	5	1	1	2		3	4	5	2		2	
	Virginiamycin S						1	7	6	9	2		

* The resistances of the four erythromycin-inducible isolates have been given in the fully induced state. Sensitive strains are inhibited by <0·1 μg/ml of erythromycin and clindamycin and by 2–16 μg/ml of virginiamycin S.

propionibacterial carriage in those patients who had previously been treated with oral erythromycin than in patients with no known previous exposure to erythromycin ($0{\cdot}01 > P > 0{\cdot}001$). The mean duration of therapy with clindamycin (9 months) was the same in both groups.

In total, 63 antibiotic resistant isolates were obtained from 52 subjects. There were 42 strains of *P. acnes*, 16 strains of *P. granulosum* and 5 strains of *P. avidum*. The strains were heterogenous with respect to their level of resistance to erythromycin, clindamycin and virginiamycin S (Table 2). Minimum inhibitory concentrations (MIC) of erythromycin and clindamycin were bimodally distributed. For both antibiotics there was a low level resistance group (MIC 0·5–2 μg/ml) and a high level resistance group (MIC of erythromycin ≥64 μg/ml, MIC of clindamycin ≥8 μg/ml). Fourteen isolates (22%) which were resistant to erythromycin were fully sensitive to clindamycin. With the exception of one isolate, the level of resistance to erythromycin always exceeded that to clindamycin. Forty-three (68%) isolates were resistant to ≥512 μg/ml erythromycin which represents over a thousand-fold increase in MIC compared with sensitive strains. Thirty-three (52%) isolates demonstrated constitutive $\mathrm{MLS_B}$ resistance and four demonstrated erythromycin inducible $\mathrm{MLS_B}$ resistance.

There was no association of any particular propionibacterial species or resistance phenotype with any treatment group, except that all six isolates from the clindamycin-treated patients with no previous exposure to erythromycin were identified as strains of *P. granulosum*. There was great variation in the number of resistant organisms present ($4{\cdot}0 \times 10^0$–$2{\cdot}3 \times 10^6$ Em^R propionibacteria/cm^2 skin), in the percentage of the flora demonstrating resistance (10^{-4}–100) and in the levels of resistance of the individual isolates to erythromycin, clindamycin and virginiamycin S. This is illustrated in Table 3 for the five patients responding to oral erythromycin who carried resistant organisms. Similar variation was found in the other treatment groups.

TABLE 3. Phenotypes of erythromycin resistant propionibacteria from patients responding to oral erythromycin therapy

Patient	MIC μg/ml of ERY	CLD	VIRG$_S$	Species	Number (%) resistant/cm^2 skin
E1	≥512	8	32	*P. acnes*	122 (100)
E2	≥512	32	64	*P. acnes*	$3{\cdot}3 \times 10^5$ (41)
	256	0·5	4	*P. granulosum*	
	1·0	0·06	32	*P. acnes*	
E3	256	0·25	128	*P. acnes*	$1{\cdot}5 \times 10^2$ (6)
	1·0	0·5	256	*P. acnes*	
E4	≥512	32	64	*P. acnes*	6 (25)
E5	2·0	512	32	*P. granulosum*	$4{\cdot}5 \times 10^3$ (0·2)

Abbreviations: ERY, erythromycin; CLD, clindamycin, VIRG$_S$ virginiamycin S.

DISCUSSION

A much higher incidence of Em^R propionibacterial carriage was found in both erythromycin- (51%) and clindamycin-treated patients (42%) than in untreated control subjects (3%), suggesting that both antibiotics select for resistant propionibacteria. However, the incidence of Em^R propionibacterial carriage in clindamycin-treated patients who had previously been treated with oral erythromycin was three times higher than in similar patients who had no known previous exposure to erythromycin. Therefore, it appears that the use of oral erythromycin encourages the development of resistance more than does the use of topical clindamycin. This is supported by the fact that, in almost all strains, the level of erythromycin resistance exceeded the level of clindamycin resistance and, even in clindamycin-treated patients, not all erythromycin resistant strains were simultaneously resistant to clindamycin. Alternatively it is possible that the patients who had received two courses of antibiotic, i.e. oral erythromycin followed by topical clindamycin, were more likely to carry resistant propionibacteria because of the increased length of exposure to selective pressure.

This study has clearly shown an association between the carriage of Em^R propionibacteria and lack of response to oral erythromycin in acne patients. Such an association had been suggested by two previous studies which did not include a responding control group.[4,6] This finding strengthens the possibility that cutaneous propionibacteria are involved in the pathogenesis of acne, evidence for which has always been inconclusive. However, the association of resistant propionibacteria with poor therapeutic outcome does not necessarily indicate that the two factors are causally related.

A number of patients who showed an initial good therapeutic response to erythromycin, followed by a gradual worsening as treatment progressed, were found to carry resistant propionibacteria. An earlier study had shown that resistant organisms take 12–24 weeks to emerge in previously untreated subjects and even longer than this to become predominant.[14] This observation may explain the initial good response and the gradual deterioration in these patients as numbers of Em^R propionibacteria began to rise.

The degree of resistance exhibited by the $\mathrm{Em^R}$ and $\mathrm{Em^RLm^R}$ strains varied considerably. It cannot at present be predicted whether the low level resistant strains (MIC 0·5–2 mg/l) should be regarded as clinically resistant, i.e. not inhibited by concentrations of antibiotic attainable *in vivo*, because skin levels of erythromycin and clindamycin are unknown. Skin levels would be unlikely to exceed serum levels ($\leqslant 3$ μg/ml) by many fold if erythromycin were administered by the oral route. Therefore, the highly resistant strains (MIC $\geqslant 64$ μg/ml) *in vitro* should also be resistant *in vivo*. Nevertheless it is possible that highly resistant strains may be inhibited *in vivo* by concentrations of erythromycin or clindamycin achievable by topical application. This could be determined by assessing the degree of response of patients with $\mathrm{Em^R}$ or $\mathrm{Em^RLm^R}$ propionibacteria treated with topical erythromycin or clindamycin, in the absence of concomitant oral antibiotic therapy.

The majority of $\mathrm{Em^R}$ propionibacteria are phenotypically similar to $\mathrm{Em^R}$ bacteria belonging to many other genera, e.g. staphylococci and streptococci, in which co-resistance to erythromycin, clindamycin and virginiamycin S is due to ribosomal methylation coded for by mobile *erm* genes located on plasmids and/or transposons.[10] This suggests that $\mathrm{Em^R}$ in cutaneous propionibacteria has arisen by transfer of methylase genes *in vivo* from either a related organism or one that shares a similar niche. We have recently shown that $\mathrm{Em^R}$ in cutaneous propionibacteria resembles that encoded by *erm* B, a gene originally isolated in streptococci but found also in several other genera including coagulase-negative staphylococci.[11] Characterization of the resistance determinant(s) in cutaneous propionibacteria and identification of their source awaits advances in the molecular genetical analysis of these organisms.

Our findings suggest that use of oral erythromycin should be limited to those patients with no previous exposure to the drug and that therapy should be discontinued after 6 months to allow any resistant organisms that have developed to be overgrown by sensitive ones. The use of benzoyl peroxide alone for a short period (2–4 weeks) may help to eradicate these organisms.

The development and spread of $\mathrm{Em^R}$ and $\mathrm{Em^RLm^R}$ in populations of cutaneous propionibacteria threatens the future usefulness of these two antibiotics in acne therapy. To date propionibacteria have not developed appreciable resistance to the tetracyclines, which remain the antibiotics of choice for acne therapy in the forseeable future.

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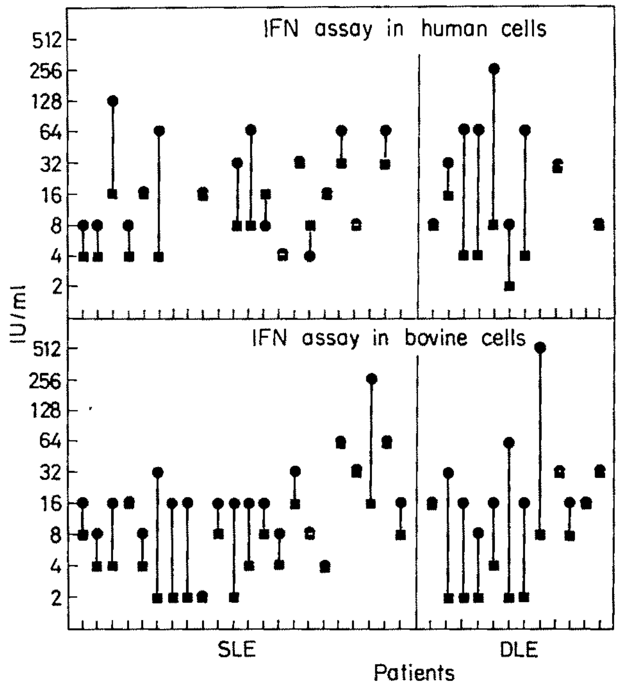

FIGURE 1. Serum IFN levels in lupus erythematosus patients before and after *in vivo* induction with dipyridamole. ■, initial level; ●, level after induction; SLE, systemic lupus erythematosus; DLE, discoid lupus erythematosus; IFN, interferon.

IFN titres obtained on human diploid lung fibroblasts were almost identical to those on primary calf kidney cells, with contradictory results in only one case. This confirms that IFN present after dipyridamole inductions is α-IFN, as it exhibits high activity in bovine cells in contrast to β-IFN and γ-IFN which are either inactive or slightly active in that cell system.[13]

DISCUSSION

It has been shown that high proportion of SLE patients have increased serum levels of α-IFN which are greatest during periods of systemic disease activity.[5,14] The significance of IFN production in SLE is unknown at present. It is possible that IFN may contribute to the pathogenesis of the disease, because of the wide range of effects it has on the immune responses. The demonstration that α-IFN can induce the *in vivo* formation of anti-DNA antibody in normal persons or in patients with SLE[15] is consistent with a pathogenic role for α-IFN. It has been found to be deposited in kidneys of SLE patients in a pattern suggesting an association with immune complexes[16] and a possible confirmation of this is the reported association between α-IFN and IgG.[17]

Since exogenous IFN is rapidly catabolized, this suggests that many SLE patients are continually producing large quantities of α-IFN. The mechanisms of induction and site of synthesis are still unknown. The IFNs are induced by a wide variety of substances other than viruses, and different cell lines are sensitive to different inducers. Early studies found the use of inducers *in vivo* unsatisfactory, because many of the substances used were too toxic in humans.[18] The safety of dipyridamole, which is among the 50 most commonly prescribed drugs in the U.S.A., overcomes this obstacle.

We administered dipyridamole as an α-IFN inducer to study the response in SLE, which is characterized by production of autoantibodies and immune complexes, and in DLE patients who are thought to express predominantly cell mediated autoimmunity, with little or no autoantibody production. The results obtained in this study suggest two types of response to induction. The high initial levels of IFN in the SLE sera are followed by a low percentage of positive responders after dipyridamole induction, whereas the more successful stimulation of DLE patients follows relatively low initial levels of IFN. This may be because SLE mononuclear cells are already overproducing α-IFN as a response to stimulation by immune complexes, antilymphocyte antibodies or other autoantibodies.[7] The mononuclear cells from DLE patients may have preserved, at least partially, their capacity to react correspondingly to induction.

It has been shown recently that DLE patients possess numbers of circulating immunoglobulin synthesizing and secreting cells, similar to those reported in active SLE, but do not demonstrate the same level of autoantibodies in their sera as do the systemic lupus patients.[3] It is probable that overproduction of IFN in DLE patients over prolonged periods of time can lead to the unchecked polyclonal B cell activation which is characteristic of SLE. This is supported by evidence that the polyclonal gammopathy observed in SLE can also be induced experimentally by α-IFN.[20,21]

Studies of factors responsible for IFN production in LE might result in better understanding of the disease and the relationship between DLE and SLE.

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Topical psoralen photochemotherapy (PUVA) and superficial radiotherapy in the treatment of chronic hand eczema

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SUMMARY

The therapeutic efficacy of conventional superficial radiotherapy and topical psoralen photochemotherapy (topical PUVA) administered over a 6 week period was compared in a double-blind study of 21 patients with chronic bilateral constitutional hand eczema. One hand was treated with conventional superficial radiotherapy and the other with topical 8-methoxypsoralen and long-wave ultraviolet light (topical PUVA). Significantly better clinical improvement was seen in superficial radiotherapy treated hands over topical PUVA treated hands after 6 weeks of treatment, but this difference was not maintained at 9 or 18 weeks. There was no significant difference in symptom severity between the two treatments after 6 weeks, but superficial radiotherapy produced significantly more symptomatic improvement at 9 and 18 weeks. Superficial radiotherapy is a less time consuming procedure than topical PUVA and leads to more rapid improvement.

Chronic constitutional hand eczema is a debilitating disorder which is often resistant to topical medication. Superficial radiotherapy is established as an effective treatment for chronic hand eczema resistant to conventional topical measures.[1,2]

Recent studies have suggested that oral psoralen photochemotherapy (PUVA) may be useful in the treatment of chronic dermatitis of the hands.[3–7] Topical PUVA avoids the potential problems of systemic administration of psoralen and has been used successfully to treat a number of conditions confined to the palms and soles, in particular psoriasis.[8] Topical PUVA has not been evaluated in the management of chronic hand eczema.

We therefore undertook to evaluate the role of topical PUVA in the treatment of chronic constitutional hand eczema. In this study we chose to compare topical PUVA with superficial radiotherapy rather than placebo because superficial radiotherapy is already established as an effective treatment.[1,2]

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METHODS

Patients

Twenty-five patients with bilateral and symmetrical chronic constitutional palmar eczema were entered into the study. All patients had chronic eczematous changes on the palms for at least 6 months with either continuous or episodic vesiculation. Twenty-one patients completed treatment. Fourteen were female and seven were male and their mean age was 52.3 years (range 19–79 years). All patients were resistant to conventional therapy with topical emollients, steroid and tar preparations. Patients with irritant and contact allergic dermatitis were excluded by a detailed history and by patch testing with the European standard battery plus additional materials where indicated.

Treatments

Patients were randomly allocated topical PUVA to one hand and superficial radiotherapy to the other using a pre-determined code. Treatment with emollients and either topical steroid or tar preparations was continued unchanged to both hands throughout the study.

The superficial radiotherapy was administered by a Dermopan II X-ray machine using a 1 mm aluminium filter and a lead applicator to maintain a focal distance of 30 cm. One hand was irradiated with 0·9 Gy at 50 KV administered on three occasions at 21 day intervals. The topical PUVA treated hand received sham radiotherapy during which the X-ray machine appeared to function normally but the power supply to the tube was interrupted such that no X-rays were received by the patient. During treatments the patient wore a protective lead apron from the neck to the knees.

Topical PUVA was administered by application of 1% 8-methoxypsoralen in an organic solvent base (Deltasoralen, Delta Laboratories Ltd, Dublin, Eire) to affected areas on the non-radiotherapy treated hand. The psoralen was applied 5 min prior to long-wave ultraviolet (UVA) exposure. A short interval between application of psoralen and UVA exposure was chosen because this was more convenient for patients and allowed more accurate application by trained nursing staff. The epidermal penetration of 8-methoxypsoralen is rapid[9] and previous studies in patients with psoriasis have shown that a short interval of 5–10 min between application of topical psoralen and exposure to UVA is as effective as longer intervals of up to 2 h.[10,11] The UVA was administered using a Blacklight HF1 unit as a light source. Exposure was started at 2 J cm^{-2} and increased gradually to a maximum of 15 J cm^{-2}. The hands were washed thoroughly with emulsifying ointment immediately after UVA exposure. Treatments were given three times a week for 6 weeks.

The superficial radiotherapy treated hand was treated with a sham PUVA procedure. This consisted of an application of the organic solvent base without psoralen 5 min prior to exposure to the light source. In addition, a 6 mm perspex filter was inserted between the hand and the light source which effectively reduced UVA transmission by more than 90%. The procedure was carried out in such a way that patients were unable to tell which hand had received active treatment.

Assessments

Patients were assessed by two observers (R.S-D and M.G) who were unaware of the treatment status of each hand until the codes were broken at the end of the study. Assessments were made prior to treatment and at 6, 9, and 18 weeks after starting treatment. A severity grade was assigned for each hand: Grade 0, normal skin; Grade 1, mild scaling and erythema; Grade 2, moderate scaling, erythema and shallow fissures; Grade 3, severe scaling, erythema and deep bleeding fissures; Grade 4, active pomphlyx. In addition the patients were asked to indicate the severity of symptoms on a 10 cm linear analogue scale.

Statistical analysis was by Wilcoxon ranked pairs tests.

RESULTS

Of the patients entered into the study, four patients failed to complete the treatments and to attend for subsequent follow up. Of the patients who completed treatment, assessments were obtained for all 21 patients at 6 and 9 weeks and for 20 patients at 18 weeks. One patient failed to attend for the 18 week follow up assessment.

The mean clinical severity scores for superficial radiotherapy and topical PUVA treated hands are shown in Figure 1. There were significant improvements from pre-treatment scores for both treatment modalities at the 6, 9 and 18 week assessments. Superficial radiotherapy was significantly better than topical PUVA at 6 weeks ($P=0{\cdot}014$) but this difference was not maintained at the 9 and 18 week assessments.

The mean symptom severity scores for superficial radiotherapy and topical PUVA are shown in Figure 2. Again there were significant improvements for both treatments at the 6, 9 and 18 week assessments. Symptom severity scores were lower for superficial X-ray treated hands than topical PUVA treated hands at 6, 9 and 18 weeks. The difference was statistically significant at 9 weeks ($P=0{\cdot}046$) and 18 weeks ($P=0{\cdot}013$), but not at the 6 week assessment.

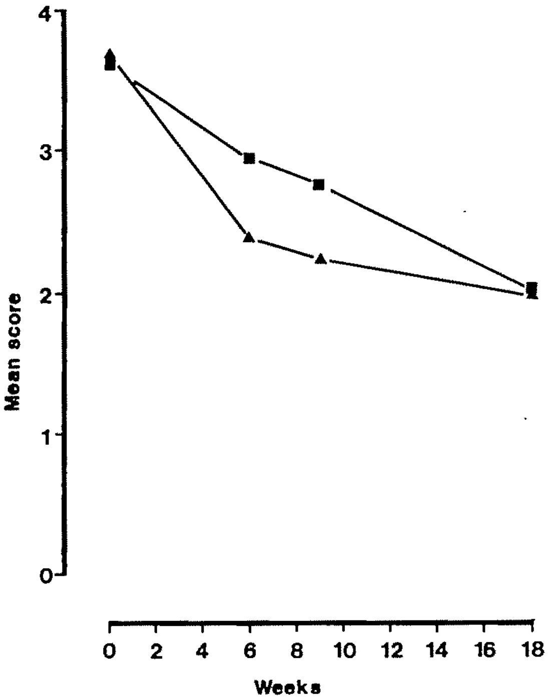

FIGURE 1. Mean clinical severity scores for superficial radiotherapy and topical PUVA treated hands. ▲——▲, superficial radiotherapy; ■——■, Topical PUVA.

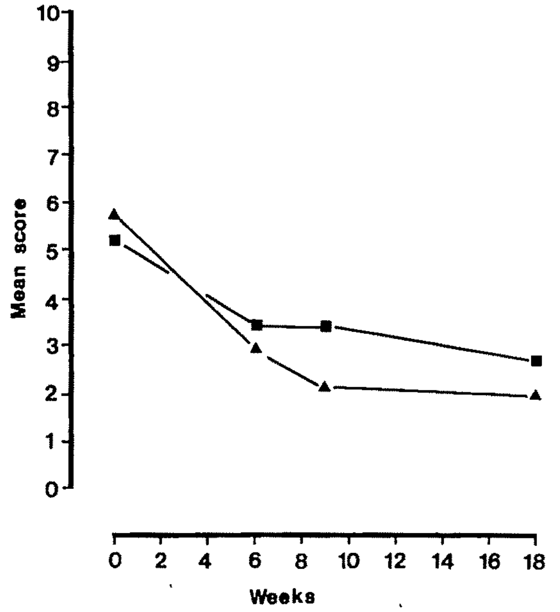

FIGURE 2. Mean symptom severity scores for superficial radiotherapy and topical PUVA treated hands. ▲——▲, superficial radiotherapy; ■——■, Topical PUVA.

DISCUSSION

Superficial radiotherapy has been shown to be of benefit in the treatment of chronic hand eczema in two placebo controlled studies. Fairris *et al.*[1] demonstrated that superficial radiotherapy gave significantly better results than placebo. The effect was maximal 6–9 weeks after initiation of treatment but persisted at 18 weeks. King and Chalmers[2] found that superficial radiotherapy gave significantly better results than placebo 7 weeks after initiation of treatment; results were better at 3 and 6 months but the differences did not reach statistical significance.

PUVA with oral psoralen has been successfully used to treat generalized constitutional eczema.[12] Uncontrolled studies have reported benefit from oral PUVA in chronic dyshidrotic hand eczema,[3–5] chronic hyperkeratotic dermatitis of the palms,[4–6] and chronic contact allergic[3,4] and irritant dermatitis.[4] One open controlled study has shown benefit from oral PUVA in patients with chronic dyshidrotic eczema of the palms.[7] Topical psoralen in PUVA treatment has been found to be effective for conditions affecting the palms and soles, such as psoriasis,[8] and avoids many of the potential problems produced by oral administration of the drug. The use of topical PUVA therapy in the treatment of chronic hand eczema has not previously been evaluated.

We have shown that topical PUVA and superficial radiotherapy are both effective over a period of 2 months in the treatment of chronic constitutional hand eczema. Improvement was more rapid with superficial radiotherapy. Superficial radiotherapy produced a more significant improvement in symptom severity than topical PUVA at 9 and 18 weeks after the start of treatment. Safe guidelines have been established for the use of superficial radiotherapy in benign dermatoses.[13] It is recommended that the total lifetime dose of conventional superficial radiotherapy should not exceed nine Gy (approximately three courses of the regime used in this study).[13] This provides a substantial margin of safety particularly for relatively radio-insensitive sites such as the palms and soles.

The frequent attendance required for topical PUVA therapy caused some difficulty for several of our patients and this could lead to poor compliance with treatment. The application of the psoralens in an organic solvent base was painful in some patients with fissures of the skin though only transiently, but this discomfort could reduce compliance and the solvent base may have a deleterious effect on dry eczema. This might be avoided by use of an alternative vehicle for administering the psoralen.

The advantages of superficial radiotherapy over topical PUVA make superficial radiotherapy a better choice for treating chronic hand eczema. However, topical PUVA is a useful alternative treatment and should be considered where superficial radiotherapy is not available or where the maximum lifetime dose of superficial X-rays have been given.

ACKNOWLEDGMENTS

We would like to thank Mrs Denise Barker and Mrs Christine Sleight for administering the superficial radiotherapy procedures.

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Relapse rates in moderately severe chronic psoriasis treated with cyclosporin A

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SUMMARY

Seventeen patients with chronic psoriasis were given cyclosporin A (CsA) 5 mg/kg per day. Twelve patients cleared within 3 months and their relapse rate, 41% at 6 months, was not significantly different from that previously reported with dithranol or PUVA. This pilot study also suggests that continuing CsA for up to 4 weeks after clinical clearance confers no advantage with regard to relapse. Significant adverse effects on renal function and blood pressure did not occur.

Cyclosporin A (CsA) is now established as an effective systemic treatment for clearing psoriasis.[1–3] It has been claimed that relapse occurs rapidly after cessation of the drug,[3,4] but this could be explained by the natural history of the very severe psoriasis in those patients.[5] If clearance were followed by a reasonable period of remission and toxicity was negligible, moderately severe psoriasis could be managed with infrequent short courses of CsA. We carried out a pilot study of relapse in psoriasis as it commonly presents in patients with a less severe disease. Since there is evidence that relapse is more rapid after a short course of treatment (Shuster, unpublished observation), we continued the treatment in some of the patients for 2 or 4 weeks after the point of clinical clearance. This also helped to eliminate any doubt about clearance as judged clinically.

METHODS

Seventeen patients (eight female, nine male, age range 19–56 years, mean duration of disease 14·5 years ± 1·52 SEM) from whom informed consent was obtained, were chosen for study. They had chronic psoriasis and the main criterion for inclusion was that they should not have had more than two attacks of psoriasis severe enough to merit hospital treatment in any previous year. The mean extent of the rash estimated by the rule of nines[6] was 22·4% ± 4·8 (SEM). All patients were normotensive with normal haematological and biochemical profiles including creatinine clearance. None were taking drugs likely to interact with CsA and women of child-bearing age were using effective contraception. Any previous treatment was stopped prior to the

TABLE 1. Patients who failed to clear after 3 months on treatment

Area at start	Area after three months' treatment
83%	5%
44%	36%
44%	10%
7%	1%
3%	1%
Mean 36·2 ± 14·6	

trial; systemic treatment at least 4 weeks previously and topical treatment at least 2 weeks previously. CsA was given as an oral suspension in a single evening dose of 5 mg/kg per day. Patients were seen at 2 to 4 weekly intervals and at each visit, the extent of the rash was estimated and blood pressure, haematological and biochemical profiles measured as well as trough (12 h after evening dose) levels of CsA in whole blood. The endpoint was complete clearance of the rash as assessed by inspection and palpation. If clearance had not been achieved in 3 months, the patients were regarded as failures for this study and took no further part in the trial though they will be described in more detail later. At the point of clearance patients were randomly allocated

TABLE 2.

Group	Patient No.	Time to clear (weeks)	Mean whole blood CsA level (ng/ml)	Time to develop new lesions (days)	Relapse, i.e. time to reach 50% original area involved (weeks)
(i)	1	15*	301	11	12
	2	10	333	3	—
	3	11	194	25	—
	4	10	(117)	16	12
Mean (SEM)		11·25 (±0·9)		13·75 (±4·6)	
(ii)	5	5	274	5	—
	6	12	198	3	20
	7	10	319	2	20
	8	8	139	2	—
Mean (SEM)		8·75 (±1·49)		3·0 (±0·7)	
(iii)	9	7	162	5	—
	10	12	217	31	—
	11	6	298	18	12
	12	7	332	6	—
Mean (SEM)		8·0 (±1·35)		15·0 (±6·0)	

* 3 week treatment free interval for side effects.

( ) Serum CsA level.

– Not relapsed at 6 months.

to one of three groups: (i) in which CsA was stopped at the point of clinical clearance, (ii) in which it was continued for 2 weeks after the point of clinical clearance, and (iii) in which it was continued for four weeks after the point of clinical clearance.

Following cessation of treatment patients were examined weekly to start with and later fortnightly and monthly. Relapse was defined as return of the rash to 50% of the area it involved before treatment.[7] The date of appearance of the first new lesions was also noted.

RESULTS

Clearance

Twelve of the 17 cleared in a mean of 9·25 ± 0·8 (SEM) weeks. All five who had not cleared in three months had nevertheless improved (Table 1).

Relapse

At 6 months, five out of 12 patients (41%) had relapsed (see Table 2) leaving seven patients (59%) who had not relapsed by our definition. Time of appearance of first lesions after stopping CsA is shown in Table 2. Once a new lesion has appeared there may be a slow extension or a rapid explosion to full relapse. The time taken, although apparently shorter in Group (ii) than in Groups (i) and (iii), is variable and more patients are needed before we can assign any clinical importance to these particular results. We found no relationship between the time taken to clear the rash and relapse rate (data not shown).

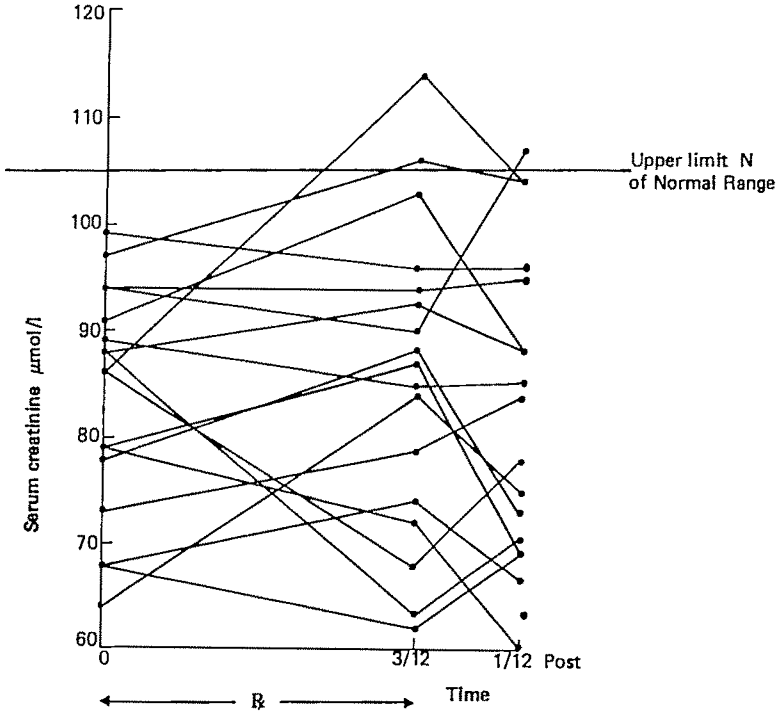

FIGURE 1. Serum creatinine during Cyclosporin A treatment.

The five patients who failed to clear were not significantly different from those who cleared with regard to age, duration of psoriasis or mean whole blood trough CsA levels, but they did have a significantly greater area of skin involved initially (mean 36·2 ± 14·6 SEM compared with 16·75% ± 2·5 SEM for those who cleared). They possibly represented the worst end of the spectrum of clinical severity in the study.

The unwanted effects were hypertrichosis (6), paraesthesiae (4), dyspepsia (2), folliculitis or increase in acne (4), gum hypertrophy (1) and amenorrhoea (1). There was no clinically significant rise in blood pressure. Five of the 17 showed a 10% rise in serum creatinine over the study (Fig. 1), but only in two did the absolute value exceed the upper limit of the normal range for the laboratory and this fell to normal within a month of stopping treatment.

DISCUSSION

Our findings show that common psoriasis requiring hospital treatment responds to CsA without a rapid relapse after cessation of therapy. In our previous studies of response to PUVA and dithranol, where the same criteria of response and relapse were used, life tables showed that the chance of having relapse at 6 months was 45% for PUVA and 55% for dithranol.[7,8] Although the numbers in our present pilot study of CsA are too small for confidence, for the group as a whole a 41% chance of relapse at 6 months is of the same order as with these other treatments. This supports our suggestion that the reason for the high relapse rates with CsA[3,4] previously reported was the severity and natural history of the disease in those patients. We are now carrying out a full scale controlled comparison of CsA with dithranol. If this study confirms our present findings it might be reasonable to use CsA in patients with moderately severe psoriasis who could be controlled with negligible side effects by infrequent short courses of the drug.

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Meta-analysis of placebo-controlled studies of the efficacy of Epogam in the treatment of atopic eczema. Relationship between plasma essential fatty acid changes and clinical response

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SUMMARY

Gamma-linolenic acid in the form of a particular variety of evening primrose oil (Epogam) has been reported of value in the treatment of atopic eczema. Nine controlled trials of evening primrose oil were performed in eight centres. Four of the trials were parallel and five cross-over. Doctors and patients assessed the severity of eczema by scoring measures of inflammation, dryness, scaliness, pruritus and overall skin involvement. Individual symptom scores were combined to give a single global score at each assessment point. In the analysis of the parallel studies, both patient and doctor scores showed a highly significant improvement over baseline ($P < 0\cdot0001$) due to Epogam: for both scores the effect of Epogam was significantly better than placebo. Similar results were obtained on analysis of the cross-over trials, but in this case the difference between Epogam and placebo in the doctors' global score, although in favour of Epogam, failed to reach significance. The effects on itch were particularly striking. There was no placebo response to this symptom, whereas there was a substantial and highly significant response to Epogam ($P < 0\cdot0001$). When the improvements, or otherwise, in clinical condition were related to changes in plasma levels of dihomogammalinolenic and arachidonic acids, it was

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found that there was a positive correlation between an improvement in clinical score and a rise in the fatty acid levels.

Hansen[1-4] was the first to suggest that atopic eczema might be related to abnormal essential fatty acid intake or metabolism. A recent study has shown that the main dietary essential fatty acid (EFA), linoleic acid, is somewhat elevated in the plasma phospholipids of adults with atopic eczema, whereas levels of its metabolites, gamma-linolenic acid (GLA), dihomogammalinolenic acid (DGLA) and arachidonic acid (AA) are significantly reduced.[5] This observation has been confirmed in children with atopic dermatitis and it has also been shown that elevated linoleic acid levels in cord blood phospholipids are positively associated with IgE concentrations.[6] This suggests that in atopic eczema there may be some impairment of the conversion of linoleic acid to GLA. GLA is found in a selected variety of evening primrose oil (Epogam) and it has been observed that Epogam could produce significant improvement in patients with atopic eczema.[7]

Clinical trials in atopic eczema are notoriously difficult to carry out, because of the inherent variability of the clinical state, the subjective nature of assessment and a large placebo response. As a result of these problems, the numbers of patients available in one centre are often insufficient to allow demonstration of a statistically significant effect, even of agents which are widely acknowledged by clinicians to be therapeutically valuable. These problems also occur in other areas of medicine. As a result, statistical techniques (sometimes known as meta-analysis) have been developed for combining the results from several different clinical trials in order to provide greater statistical power.[8-11] In order to qualify for such analysis the trials must be similar and the trends in the results of the individual trials must also be similar.

The present analysis combines the results of the two Wright and Burton studies (adults and children) with seven other investigations.

METHODS

The trial designs are summarized in Table 1. All the patients had a skin disease which was diagnosed clinically as being atopic eczema; in all patients this disease had begun in infancy or childhood; and in all patients there was a family history of atopic disorders. Duration of active treatment was 12 weeks, except in the Nottingham trial in which it was 8 weeks and in the Bologna trial where it was 4 weeks. All the trials were randomized, double-blind, and placebo-controlled. Three hundred and eleven patients took part in the trials, 167 of them being female. Their ages ranged from 1 to 60 years.

In each trial the GLA was administered in the form of 500 mg capsules of Epogam. There are many different wild and cultivated strains of the evening primrose (*Oenothera biennis*) from which the seed oil containing GLA can be isolated. These may vary considerably in GLA content. Epogam is the seed oil from strains selected and bred to yield oil of constant composition.

In all the trials the patients were currently receiving the best available topical treatment for their grade of eczema. The exception was the Turku trial where patients stopped their existing medication and were each given standard emollient and mild topical steroid creams which they could use in unlimited quantities. Use of these preparations was one measure of outcome in this trial. In each trial the actual parameters studied were slightly different but always included measures of inflammation, dryness, scaliness, and overall skin involvement. In six of the trials

TABLE 1. Clinical trials analysed in this study

Study	Study design	Duration (weeks)	Wash-out (weeks)	Number of clinical assessments		Treatment*	Number of patients entered						Number of patients taking potent steroids†
				By clinician	By patient		Dose (capsules/day)					Total	
							2	4	6	8	12		
Burton (adults)	X-over	12+12	0	3	4	Epogam	0	10	0	10	9	29	2
						Placebo	0	10	0	10	11	31	3
Burton (children)	X-over	12+12	0	3	4	Epogam	9	9	0	0	0	18	0
						Placebo	9	12	0	0	0	21	1
Gould	X-over	12+12	4	5	3	Epogam	0	0	5	15	0	20	1
						Placebo	0	0	7	15	0	22	0
Allen	X-over	8+8	0	5	6	Epogam	0	0	11	12	0	23	1
						Placebo	0	0	12	12	0	24	3
Wexler	X-over	12+12	0	6	5	Epogam	0	4	1	4	0	9	3
						Placebo	0	3	1	1	0	5	0
Jansen	Parallel	8	—	6	0	Epogam	0	0	0	14	0	14	0
						Placebo	0	0	0	11	0	11	0
Holt	Parallel	12	—	5	3	Epogam	0	0	0	19	0	19	0
						Placebo	0	0	0	18	0	18	0
Meigel	Parallel	12	—	5	0	Epogam	0	0	0	0	18	18	10
						Placebo	0	0	0	0	7	7	5
Bordoni	Parallel	4	—	10	0	Epogam	0	0	10	0	0	10	0
						Placebo	0	0	12	0	0	12	0

* First treatment received if X-over study.

† Patient on potent topical steroids, or systemic steroids.

(excluding Meigel and Jansen) patients also assessed their own condition on similar scales and also included itch. In the Meigel, Jansen and Bordoni trials, itch was assessed by the doctor in consultation with patients or parents. There were thus three sets of scores: clinician, patient and itch.

Four of the studies were originally designed as parallel studies. The first phase of each cross-over trial could also be treated as a parallel study. In order to transform the data into a format which could be handled in a common manner, the original information from the trials was treated as follows. Absence of any feature of eczema was classed as a score of 0, while a maximum score on all the clinical features assessed in the trial was classed as 100. The total possible score of 100 was equally divided between the number of parameters assessed in the trial. For example, if four parameters were assessed, each was given a maximum possible score of 25, while if 10 parameters were assessed each was given a maximum score of 10. The actual scores recorded by the patients and doctors were then transformed in accordance with the above system so that at each assessment point, the severity of an individual's eczema could be represented by a single number, termed the global severity score. Itch was assessed separately.

Statistical computer program BMDP 4F[12] was used to test whether there were significant differences between the patterns of results in the different trials using the Mantel–Haenszel test of homogeneity.[10] Each patient was classed as improved or not improved with reference to baseline at the end of the parallel trials and at the end of the first parallel phase of the cross-over trials. There were no significant differences between the patterns of results ($P > 0{\cdot}1$). Therefore, using the same computer program, the results of all the trials were combined.

Parallel studies and first phases of cross-over studies.

Four of the studies were originally designed as parallel studies. The first phase of a cross-over study can also be treated as a parallel study. The data were analysed as follows: Two assessment points were considered. For all trials the last assessment point was based on the last available data for that patient. For all the trials except Bordoni, the first assessment was based on the initial 3 or 4 week assessment point. Because the Bordoni trial in young children lasted only 4 weeks, first assessment points were not included. The change in score from starting Epogam or placebo to the first or last assessment point was noted, and the significance of these changes from baseline was assessed separately for each treatment by Wilcoxon signed ranks test. The changes in score in response to Epogam were compared to the changes in score in response to placebo by Mann-Whitney *U*-test. The size of the response was expressed in percentage terms by expressing the change in response to either Epogam or placebo as a percentage of the starting severity score.

Cross-over studies

Five of the studies were designed as cross-over studies. The possibility of a carry over effect of Epogam was tested using Gart's modification of a Chi-squared analysis,[13] which compares the results in the patients using placebo first with those in patients using Epogam first. This showed the presence of highly significant carry over effects at the first assessment point after cross-over. At the last assessment point, the carry over effect remained significant for patient and doctor global scores but not for itch. The carry-over effect is shown in Fig. 1. Patients treated with Epogam first showed little or no deterioration when switched to placebo, while those treated with placebo first showed a further improvement on switching to Epogam.

When there is no carry over effect of treatment, it is usual to estimate the difference between active and placebo by comparing the results at the end of the first and second phases of

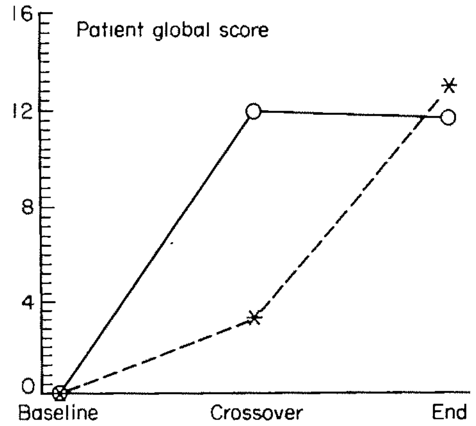

FIGURE 1. Quantitative response in patients' global score in the cross-over trials. Patients who received Epogam first and those who received placebo first are shown separately. Epogam first —○—; placebo first ---*---.

treatment. When there are strong carry over effects, as in this case, then this form of analysis is inappropriate. Instead, for the Epogam-treated patients, the score at the first and last assessments during each treatment period was compared with the starting score for that treatment period (i.e. either baseline or cross-over point depending on the treatment sequence). The statistical significance of the change in score was compared using Wilcoxon signed ranks test. The placebo-treated patients were treated similarly. The difference in response to Epogam and to placebo was then compared by analysing the results by Mann-Whitney U-test. The size of the response was expressed in percentage terms by expressing the change in response to either Epogam or placebo as a percentage of the starting severity score.

Odds ratio analyses

In the parallel studies and the first phase of the cross-over studies, a reduction in score between the start of treatment and either the first or last assessment points was classed as an improvement, while no change in score or an increase in score were classed as a deterioration. The odds ratio is the ratio of the odds of improvement on Epogam to the odds of improvement on placebo. An odds ratio of greater than 1·0 shows that proportionally more patients improved on Epogam than on placebo. Statistical computer program BMDP 4F was used to give an overall estimate of the odds ratio, together with an estimate of its statistical significance and 95% confidence intervals.

In the cross-over studies, the score at the first and second assessment points on Epogam was compared with the score at the first and second assessment points on placebo. When this severity score was less on Epogam than on placebo, the patient was said to be better on Epogam. When the scores were equal, or when the severity score was less on placebo than on Epogam, the patient was said to be better on placebo. Again the odds in favour of Epogam were calculated using statistical computer program BMDP 4F, analysing the two phases of treatment separately. This time the comparisons between Epogam and placebo were for the same patient rather than for different patients as in the parallel studies.

All significance tests quoted in the results are based upon 2-tail tests and are therefore quoted as $2P =$ or $2P <$.

Oral and potent topical steroid use

Twenty-nine patients were using oral steroids or potent topical steroids. Since there are theoretical reasons why these might interfere with treatment using EFA's (inhibition of conversion of EFAs to prostaglandins), the analyses were repeated with and without these patients.

Blood sample analysis

Plasma samples were collected from some of the adult patients at the beginning of the trial and at the end of the active and placebo treatment periods in the trials conducted by Burton, Jansen, Gould and Allen. All available plasma samples were used in the analyses. Blood was taken into EDTA tubes and centrifuged. The plasma was separated and frozen and at the end of the trial, shipped frozen by air to the Efamol Research Institute where analyses were carried out under code. The biochemists performing the analyses were unaware of which were baseline and which were active treatment samples until all the analyses had been carried out and the codes had been broken.

The plasma samples were analysed as described previously.[5,14] Samples were initially extracted with chloroform–methanol. The extract was filtered through sodium sulphate, evaporated to dryness and taken up again in chloroform–methanol. The various lipid fractions were separated by thin-layer chromatography on silica gel plates. The phospholipid fraction, whose composition is resistant to immediate changes in response to meals but which is a sensitive indicator of long term essential fatty acid changes, was methylated using boron trifluoride–methanol. The resulting methyl esters of the fatty acids were separated and measured using a Hewlett–Packard 5880 gas chromatograph with automatic integration. Peaks were identified by comparison with standard fatty acid methyl esters obtained from Nu-Chek Prep Inc, Elysian, Minnesota, U.S.A. Samples and clinician scores were available for 86 patients at the end of either active or placebo periods. Patient scores were available for 78 samples since patient assessment was not used in the Jansen trial.

For each individual the changes in plasma phospholipid dihomogammalinolenic acid (DGLA) and arachidonic acid (AA) were calculated from the baseline to the end of the active or placebo treatment period. The sums of the two changes were also calculated. The corresponding changes in global score from the baseline to the end of active or placebo treatment were also noted. Correlation analysis was used to test for relationships between fatty acid changes and clinical response.

RESULTS

Parallel studies—quantitative analyses

The results are shown in Table 2. Epogam caused a significant improvement from baseline on all three scores at both time points. At the last assessment, all the significance levels showed $2P < 0{\cdot}0001$. None of the improvements seen on placebo was significant at the first time point and only one of the three was significant at the last assessment. At the first assessment, the response to Epogam was significantly greater than the response to placebo for two of the three scores while at the last assessment Epogam was significantly better on all three scores.

The effect of Epogam was progressive. The size of the effect of Epogam and the differences between the effects of Epogam and placebo were consistently greater at the last assessment point than at the first. The effect on itch was particularly clear with an overall average 25·2% improvement on Epogam and a slight deterioration on placebo.

TABLE 2. Summary of the changes from baseline score in response to Epogam and to placebo. A plus sign shows improvement and minus sign deterioration. The significances of the changes from baseline for active and placebo groups and the significances of the differences between the Epogam and placebo responses are also shown. The figures in brackets in the Change column indicate the size of the response as a percentage of the baseline score. Data based on all the parallel trials and first phases of all the cross-over trials

Assessment	Time	*n*	Change on Epogam + SEM and (%)	Significance of change from base-line to Epogam $2P =$	*n*	Change on placebo + SEM and (%)	Significance of change from base-line to placebo $2P =$	Mean difference between treatments	Significance of difference between treatments
Patient global	First	103	4·2 ± 1·9 (8·9%)	0·0260	93	−0·4 ± 2·3 (−0·9%)	0·9250	4·6 ± 3·0	0·1138
	Last	112	10·3 ± 2·4 (21.7%)	< 0·0001	104	3·7 ± 2·6 (8·6%)	0·1934	6·6 ± 3·5	0·0388
Clinician global	First	141	5·7 ± 1·3 (11·7%)	< 0·0001	112	0·5 ± 1·3 (1.2%)	0·9277	5·2 ± 1·9	0·0074
	Last	154	11·2 ± 1·7 (23·0%)	< 0·0001	132	5·9 ± 1·5 (13·9%)	0·0001	5·3 ± 2·3	0·0110
Itch	First	143	6·3 ± 2·4 (10·5%)	0·0274	113	−2·6 ± 2·7 (−5·9%)	0·4067	8·9 ± 3·6	0·0552
	Last	154	15·1 ± 2·6 (25·2%)	< 0·0001	132	−0·6 ± 2·6 (−1·4%)	0·9295	15·7 ± 3·7	< 0·0001

TABLE 3. Summary of the response rates and odds ratios in favour of Epogam for the first and last time period assessment for the overall global score, the patient's and clinician's global score, and patient itch measurements in parallel studies

Assessment	Time	Treatment	Improved	Not improved	Odds ratio pro Epogam	95% confidence interval	Significance
Patient Global	First	Epogam	60 (58%)	43 (42%)	1·6	0·9 to 2·9	0·1361
		Placebo	44 (47%)	49 (53%)			
	Last	Epogam	75 (67%)	37 (33%)	2·2	1·2 to 3·9	0·0117
		Placebo	52 (50%)	52 (50%)			
Clinician Global	First	Epogam	77 (55%)	64 (45%)	1·6	0·9 to 2·8	0·0797
		Placebo	45 (40%)	67 (60%)			
	Last	Epogam	111 (72%)	43 (28%)	1·7	1·0 to 2·9	0·0591
		Placebo	80 (61%)	52 (39%)			
Itch	First	Epogam	65 (46%)	78 (54%)	1·6	0·8 to 2·5	0·01137
		Placebo	41 (36%)	72 (64%)			
	Last	Epogam	97 (63%)	57 (37%)	2·7	1·5 to 4·5	0·0001
		Placebo	51 (39%)	81 (61%)			

Data based on all parallel trials and the first phase of all crossover trials.

Parallel studies—odds ratio analyses

The results are shown in Table 3. For all three scores, at both first and last assessment points, Epogam is better than placebo. Both the patient global score and the itch score are significantly in favour of Epogam, the itch score at $P < 0·0001$. The degree of significance in favour of Epogam is always greater for the last assessment point than the first, indicating a progressively increasing difference between Epogam and placebo.

Cross-over studies—quantitative analyses

The results are shown in Table 4. Epogam produced a significant improvement over the starting value at the first and last assessments for all three scores. Placebo had no significant effect on any scores at the first assessment point and on only one of the three at the second assessment point. The effect of Epogam was greater than the effect of placebo for all three scores at both assessment points. For the patient and the itch scores the differences between Epogam and placebo were significant. The effect on itch was again particularly clear, with an average 24·2% improvement on Epogam and a 3·1% deterioration on placebo.

Cross-over studies—odds ratio analyses

The results are shown in Table 5. Gart's test for carry over effects shows that these were substantial. The carry over effect was significant for all three scores at the first assessment point and for two of the three at the last assessment point. The carry over effect is also shown in Figure 1. The two treatment sequences, Epogam–placebo and placebo–Epogam, are shown separately in Table 5.

In the patients who used Epogam first, there was either only a small deterioration, no change, or a further improvement on switching to placebo, indicating a prolonged effect of Epogam. As a consequence, none of the differences between the Epogam and placebo effects was statistically

TABLE 4. Comparison of the quantitative change produced by Epogam and placebo in the five cross-over trials. A plus sign shows improvement and a minus sign deterioration. Improvements over the starting value were analysed by Wilcoxon's signed rank test and differences between Epogam and placebo by Mann–Whitney *U*-test

				Improvement with Epogam			Improvement with placebo			Difference between treatments	
Assessment	Time	Number of patients receiving both treatments	Baseline mean (both sequences)	Mean ± SEM	% Change from baseline	Significance 2*P* =	Mean ± SEM	% Change from baseline	Significance 2*P* =	Mean ± SEM	Significance 2*P* =
Patient Global	First	133	44·3	5·1 ± 1·6	(11·5%)	0·0009	−0·8 ± 1·8	(−1·8%)	0·5134	5·9 ± 2·6	0·5134
	Last	170	46·2	11·0 ± 1·8	(23·8%)	< 0·0001	1·6 ± 1·9	(3·5%)	0·5419	9·4 ± 3·1	0·0013
Clinician Global	First	129	43·7	5·4 ± 1·4	(12·3%)	0·0003	1·6 ± 1·6	(3·7%)	0·3925	3·7 ± 2·2	0·1001
	Last	170	43·7	9·8 ± 1·6	(22·4%)	< 0·0001	6·1 ± 1·8	(14·0%)	0·0003	3·7 ± 2·9	0·1486
Itch	First	133	47·8	5·5 ± 2·0	(11·5%)	0·0137	−2·5 ± 2·1	(−5·2%)	0·2268	8·0 ± 3·2	0·0190
	Last	170	48·7	11·8 ± 2·15	(24·2%)	< 0·0001	−1·5 ± 2·2	(−3·1%)	0·6924	13·4 ± 3·7	0·0011

TABLE 5. Results of the cross-over trials showing which treatment was found to be better when the end of active and end of placebo periods were compared. Because the carry-over effect was substantial, as shown by the significance of Gart's carry over test, the results are shown separately for those who took Epogam first and those who took placebo first

		Patients receiving Epogam first						Patients receiving placebo first						
Assessment	Time	*n*	Epogam better	No difference	Placebo better	Odds* pro-Epogam	2*P*=	*n*	Epogam better	No difference	Placebo better	Odds* pro-Epogam	2*P*=	Significance of carry-over 2*P*=
Patient Global	First	67	27 (40%)	2 (3%)	38 (57%)	0·71	0·2150	66	43 (65%)	4 (6%)	19 (29%)	2·26	0·0035	0·0002
	Last	89	43 (48%)	5 (6%)	41 (46%)	1·05	0·9124	81	58 (72%)	3 (4%)	20 (25%)	2·90	<0·0001	0·0022
Clinician Global	First	65	20 (31%)	3 (5%)	42 (65%)	0·48	0·0075	64	47 (73%)	6 (9%)	11 (17%)	4·27	<0·0001	<0·0001
	Last	89	32 (36%)	8 (9%)	49 (55%)	0·65	0·0750	81	46 (57%)	1 (1%)	34 (42%)	1·35	0·2186	0·0220
Itch	First	67	25 (37%)	7 (10%)	35 (52%)	0·71	0·2460	66	36 (54%)	10 (15%)	20 (41%)	1·80	0·0455	0·0143
	Last	89	42 (47%)	10 (11%)	37 (42%)	1·14	0·6528	81	49 (60%)	9 (11%)	23 (28%)	2·13	0·0032	0·0609

* $\frac{\%\text{ in favour of Epogam}}{\%\text{ in favour of placebo}}$.

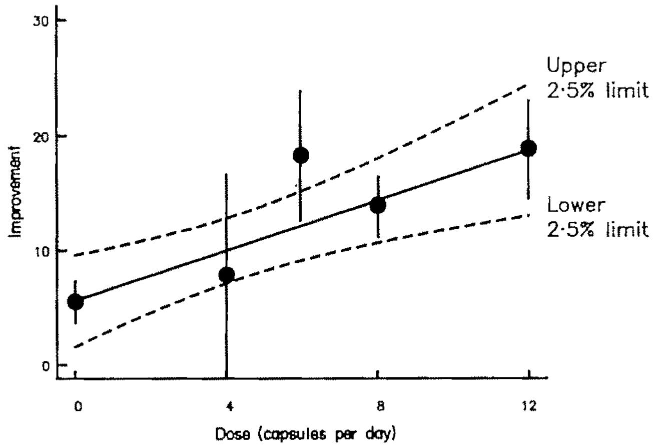

FIGURE 2. The effect of dose on the overall global score (195 patients, $2P = 0{\cdot}0001$). The figure shows the regression line and the 95% confidence intervals for the position of the line. Ninety-two patients received 0 capsules per day. Eight patients received four capsules. Ten patients received six capsules. Sixty-five patients received eight capsules and twenty patients received 12 capsules per day. The bars show SEM.

significantly in favour of Epogam. In contrast, most of the patients who received placebo first showed a substantial improvement on switching to Epogam. For this treatment sequence, all of the three scores at both assessment points were in favour of Epogam and five of the six scores were significantly so.

Effects in relation to dose

Because of the great variability in weight of children of different ages and the likelihood of differing essential fatty acid requirements in pre-pubertal children, an attempt to evaluate the effect of dose on clinical response was based on the adult studies only. Because of the significant carry over effects, only the parallel studies and not the second phases of the cross-over studies were included. The size of the change in the overall global score was compared in the placebo group (92 patients) and in those adults who received four capsules/day (eight patients), six capsules per day (10 patients), eight capsules per day (65 patients) and 12 capsules per day (20 patients). The results are shown in Figure 2 which shows the individual points, the regression line and the 95% confidence intervals for the position of the regression line. By analysis of covariance, using the study as factor and the dose as the covariate, there was a highly significant effect of dose on the response ($2P = 0{\cdot}0001$). The analysis also showed that when dose was taken into account there was no statistically significant difference between the studies.

Use of steroids

Exclusion of those patients who had been using oral steroids or the most potent topical steroids concomitantly made no difference to the overall pattern of the response or to which results were significant, or to the order of magnitude of the levels of significance.

Plasma fatty acids and clinical response

Figure 3 shows the relationship between the change in clinical score assessed by patients and the change in plasma dihomogammalinolenic acid and arachidonic acid. Correlation analysis showed that there was a rather weak but significant positive correlation ($r = +0{\cdot}26$, $P = 0{\cdot}022$,

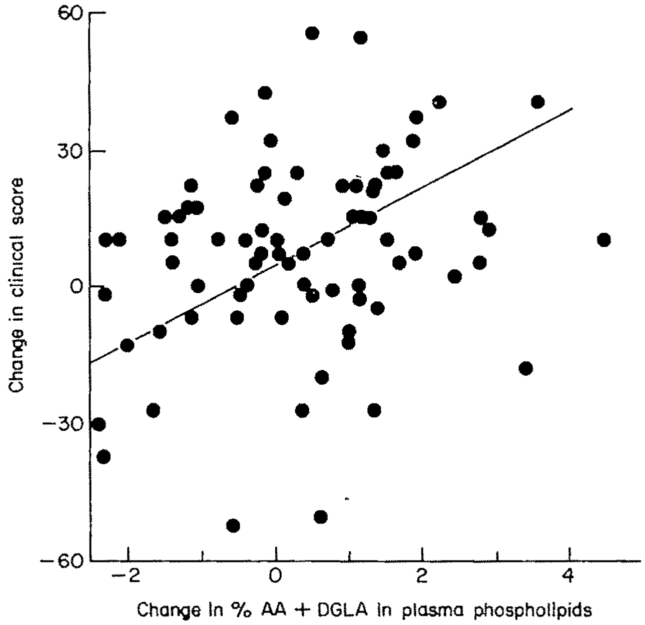

FIGURE 3. Eczema score and plasma fatty acids—patient assessment. The relationship between changes from baseline in the clinical score and in the concentration (mg/100 mg total lipid present) of dihomogammalinolenic acid (DGLA) and arachidonic acid (AA) in plasma phospholipids. A positive figure for the clinical score indicates an improvement. $r = 0{\cdot}26$; $P = 0{\cdot}022$.

$n = 78$). There was a similar positive relationship for the doctor assessments but this did not reach statistical significance.

DISCUSSION

The results show that the effects of Epogam are almost always significantly better than those of placebo. Improvements during Epogam treatment and differences between the effects of Epogam and placebo were often highly significant ($2P < 0{\cdot}01$). They were also consistently greater at the last assessment point than at the first, indicating a progressive effect of Epogam. The cross-over studies showed the presence of a significant carry over effect from Epogam which is in itself an indication of efficacy. There was a significant dose/response relationship, with higher doses producing a greater clinical effect.

There was no overall effect of placebo on itch, suggesting that itch in atopic eczema has a biological rather than a psychogenic origin.

The overall average improvements with Epogam in percentage terms were in the 23–26% range. Patients in the study were concomitantly receiving what was regarded as the best available therapy for their grade of eczema. The improvements seen in the Epogam group therefore represent improvements over and above the best that can be achieved using conventional available therapy.

Arachidonic acid (AA) and dihomogammalinolenic acid (DGLA) are the two major metabolites of GLA in the plasma phospholipids. GLA is very rapidly metabolized to DGLA and then DGLA is more slowly converted to AA via the rate-limiting 5-desaturase (Fig. 4).[15]

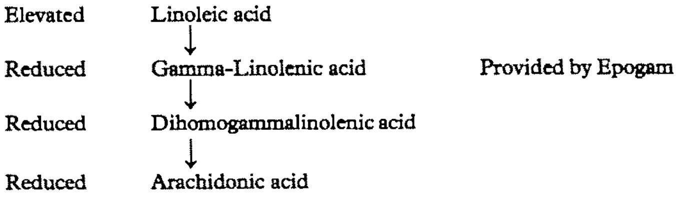

FIGURE 4. Outline of essential fatty acid metabolism in patients with atopic eczema.

Figure 3 shows that there was a weak but significant positive correlation between changes in DGLA + AA and clinical response. AA alone showed a positive correlation, DGLA alone showed a very weak and non-significant relationship. The sum of the two produced the best correlation suggesting that both contribute to the clinical response.

The results of the administration of Epogam and of the blood sample analyses support studies[1-6,16] which suggest that atopic eczema may be related to essential fatty acid metabolism. A stronger correlation between fatty acid change in plasma phospholipids and clinical effect may have been lacking because the delivery of the EFAs to the skin is likely to be affected by many factors other than the level of the fatty acid in the plasma phospholipids. This study provided no evidence as to whether a failure of plasma EFA to rise could be attributed to lack of compliance, malabsorption, or to abnormal EFA metabolism. However, there is unlikely to be a clinical response to GLA without a change in the plasma level of GLA or its principal metabolites, DGLA or AA.

There is a good deal of evidence that the products of AA are generally pro-inflammatory in the skin, while DGLA and its metabolites may have anti-inflammatory effects under certain conditions.[17] Steroids are believed to act, at least in part, by reducing the mobilization of AA from phospholipid stores and hence the availability of free AA for conversion to pro-inflammatory metabolites. In our study a rise in AA in plasma was associated with a good clinical response, while a rise in DGLA was less clearly associated with a response. The best clinical response was associated with the rise in the sum of DGLA + AA. The following hypotheses may explain this phenomena.

Firstly, skin inflammation in atopic eczema is associated with excess production of AA-derived eicosanoids, and steroids do act in part by reducing conversion of AA to eicosanoids. However, the key component of the steroid effect which produces the therapeutic response may not only be the reduction of eicosanoid levels. Instead the conservation of AA in the skin phospholipids and other lipid fractions may be important. There is increasing evidence that in most tissues the EFAs themselves have major roles which do not require their conversion to eicosanoids. The EFAs are critical components of normal membrane structure and help to determine such parameters as membrane fluidity, flexibility and permeability and the functions of membrane-bound enzymes and receptors. If eicosanoid reduction is the only mechanism of steroid action, then EFAs and steroids would be expected to interfere with one another's therapeutic effects. If AA conservation is important, then the effects of steroids and EFAs should not be antagonistic.

Secondly, a rise of DGLA may not be harmful in itself but may merely be an indication that the 5-desaturase enzyme, which converts DGLA to AA, has restricted activity. A high DGLA may therefore, simply be an indicator of low AA. Indeed, at the start of treatment the higher the level of DGLA, the lower was the level of AA ($R = -0{\cdot}29, P < 0{\cdot}18$). That DGLA itself may be

TABLE 6. Summary of the changes from baseline score in response to Epogam and to placebo in the nine trials analysed in this paper with and without the inclusion of the data from the Bamford study.[18] A plus sign indicates improvement and minus sign deterioration. The significances of the changes from baseline for active and placebo groups and the significances of the differences between the Epogam and placebo responses are also shown. The figures in brackets in the Change column indicate the size of the response as a percentage of the baseline score. Data based on all the parallel trials and first phases of all the cross-over trials

Assessment	Time	*n*	Change on Epogam ± SEM and (%)	Significance of change from base line to Epogam $2P =$	*n*	Change on placebo ± SEM and (%)	Significance of change from base-line to placebo $2P =$	Mean difference between treatments	Significance difference between treatments
Patient global	Last excluding Bamford	112	10·3 ± 2·4 (21·7%)	< 0·0001	104	3·7 ± 2·6 (8·6%)	0·1934	6·6 ± 3·5	0·0388
	Last including Bamford	177	8·0 ± 2·4 (17·4%)	< 0·0001	175	7·1 ± 2·0 (15·7%)	0·0006	0·9 ± 2·9	0·5465
Clinician global	Last excluding Bamford	154	11·2 ± 1·7 (23%)	< 0·0001	132	5·9 ± 1·5 (13·9%)	< 0·0001	5·3 ± 2·3	0·0110
	Last including Bamford	218	12·6 ± 1·3 (27·6%)	< 0·0001	204	8·9 ± 1·2 (22·1%)	< 0·0001	3·7 ± 1·8	0·0165
Itch	Last excluding Bamford	154	15·1 ± 2·6 (25·2%)	< 0·0001	132	−0·6 ± 2·6 (−1·4%)	0·9295	15·7 ± 3·7	< 0·0001
	Last including Bamford	218	11·6 ± 2·2 (21·0%)	< 0·0001	202	5·3 ± 2·2 (10·5%)	0·0147	6·3 ± 3·1	0·0296

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Dietary manipulation in childhood atopic dermatitis

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SUMMARY

The dietary habits of 73 children seen consecutively in a dermatology out-patient department were studied to ascertain the nature and frequency of dietary manipulation and to assess any potential hazards. Although most children had only mild flexural dermatitis, 71% had had significant alterations made to their diet before attendance at hospital. Almost all diets were unsupervised and only six children had seen a dietitian. Over half had been started on diets before any professional advice had been sought. Information on diet had come from media, books or magazines (51%), friends (32%), general practitioners (27%) and district nurses or health visitors (18%). The major dietary alterations included the exclusion of eggs, dairy products and food additives, and the use of soya and goat's milk. Under 10% of patients claimed benefit from these largely unsupervised diets. Harmful practices included the frequent use of unboiled, unpasteurized goat's milk, unmodified goat's milk in a baby of 4 months and the use of severely restricted diets. Three children (6%) in the diet group had been on diets which were considered by a hospital dietitian to be particularly dangerous. However, there was no evidence of growth retardation, failure to thrive or specific deficiency syndromes in the group we studied. Uncontrolled, unsupervised and often prolonged dietary alterations are commonplace in children with atopic dermatitis. This is a potentially serious health problem.

Dietary alteration has been recommended as a treatment for childhood atopic dermatitis.[1-4] However, the value of this has not been established and several authors have pointed out the potential physical, psychological and financial hazards.[3,5-11] The need for careful dietetic supervision and the appropriate selection of patients has been repeatedly stressed.[1,7-9,12-14]

However, it was our impression that many children with atopic dermatitis have been put on uncontrolled and unsupervised diets, and that parental education about atopic dermatitis has been poor. We have, therefore, attempted to assess the extent and potential hazards of this practice.

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METHODS

Seventy-three consecutive children with typical symmetrical atopic dermatitis seen in the Dermatology Department of the Leicester Royal Infirmary were studied. These were either new patients or patients under follow up attending for routine review. There were 41 boys and 32 girls. Only children under 16 years of age were included, the age range being 4 months to 15 years (mean 5·1 years). At least one parent was required to be present (usually mother or both parents). Twenty-nine (40%) of the children were of Asian origin, the remainder were white. A semi-structured questionnaire was administered by one of us (SAW).

A history including age of onset and course of the dermatitis was obtained, and the treatment regimes and need for hospital admissions recorded. The amount and potency of topical steroids used per year was assessed. Details of past medical history and any family history of atopy were noted. Each child was examined by one of us (SAW) and the percentage of body surface area involved at the time recorded. The overall severity of dermatitis was assessed retrospectively by the examining doctor on a scale of 1–5 (1—least; 5—most severe) using a combination of the history, case notes, treatment requirements and clinical examination. Parents were also asked to grade independently the average severity of their child's dermatitis on the same 1–5 scale. The height, weight and head circumference of each child was measured and plotted on standard centile charts.

Parents were questioned about occupation, height, diet (for example vegetarian) and religion. They were asked about their general views on the cause of dermatitis and whether they had any view on the role that diet might play, both generally and with specific reference to possible precipitants in their child. The sources of any such information were then examined, with particular reference to the nature of the advice obtained from health professionals. Parents were asked to give details of any dietary changes they had introduced and to state whether these were carried out before or after professional advice (if any) had been sought. They were asked if they had seen a dietitian at any time. Finally, they were asked whether they thought that diet had helped.

RESULTS

The diets of 52 of the 73 new and review children (71%) in the study group had been significantly altered before their first attendance at hospital. Dietary alteration was defined as the exclusion of at least one major item of food, previously regularly eaten, for one week or more. The incidence was greater amongst white families (35/44; 80%) than Asian families (17/29; 59%) [χ^2 $P=0{\cdot}05$]. All the white families and 22/29 (76%) of the Asian families were normally meat-eating.

Alterations made to the children's diets ranged from exclusion of minor items to complex exclusion diets. The major items excluded are shown in Table 1. Two children had supplements made to their diets (zinc in one; oil of evening primrose in another). One mother had set up a local 'allergic children's group' to exchange ideas about food allergy in dermatitis. Most parents in the diet group had experimented with the exclusion of a variety of food items at different times. Most dietary changes were carried out within 1–2 years of the onset of the dermatitis. Of those children in the diet group (total 52), five (10%) had been on diets for a period from 1 week to 1 month, 11 (21%) from between 1 and 3 months, 12 (23%) from 3 months to 1 year and 24 (46%) had been on diets for over a year.

Ninety per cent of the parents with children on diets definitely felt that the diet had been of no

TABLE 1. Major alterations made to children's diets ($n = 52$)

Excluded items	Number
Cow's milk	38
Eggs	14
Preservatives and additives	11
All cow's milk products	10
Colourings	7
Tomatoes	5
Sweets	5
Complex exclusion diets from Immunology clinic	5
All cheeses	3
Others (18 items or group of items in total)	40*
Specific Additives	
Goat's milk	24†
Soya milk	14
Zinc	1
Oil of evening primrose	1

* This includes one gluten free diet and one low salt diet.

† Only nine of 18 parents recalled boiling unpasteurized goat's milk.

benefit to the extent or severity of the dermatitis, although nine found that a single foodstuff appeared to exacerbate the condition (Table 2). The remaining 10% of parents thought that diet may have been of some help, but none had found that the eczema had improved sufficiently to suspend other therapeutic intervention. Several parents stated that, despite the lack of benefit from exclusion diets, they had not re-introduced foods through fear of exacerbating their child's dermatitis. Several parents and children remarked that the diet could be more trouble than the dermatitis, especially when the child reached school age and started to visit the homes of friends.

Several potentially harmful practices were encountered. For example, goat's milk (usually bought unpasteurized) was not boiled in approximately two-thirds of cases. Three diets were considered by a hospital dietitian to be particularly dangerous. In the first, a baby of almost

TABLE 2. Food(s) thought to cause exacerbation of eczema among the diet group $n = 9$

Food blamed	Number
Cow's milk	2
Ketchup	2
Sweets	1
Fizzy drinks	1
Colourings	1
'Fatty' foods	1
Chocolate, fizzy drinks and grapefruit	1

4 months was given unmodified and unpastueurized goat's milk despite its high solute load and infection risk. This was recommended by a district nurse and was continued for 6 weeks until the child's grandmother told her daughter that such unmodified milks were unsuitable for such a young child. In a second case a 7-month-old boy was started on a diet intended to be free of gluten, meat, all cow's milk products, eggs and all added sugar. This diet was continued for 3 months and the child was fed almost entirely on unpasteurized goat's milk and minced vegetables, in particular large amounts of carrots. The child's father was a catering manager with the local council and no professional advice had been sought. In the third case a 3-year-old boy was started on a diet free of all cow's milk products, eggs and meat and from which all added sugar and salt were excluded. This diet was continued for 18 months. Again, no professional advice had been sought. None of these three children had severe dermatitis, the first two being labelled as Grade 2 and the last as Grade 3 by the examining doctor.

Parental views as to the major causes of dermatitis are shown in Table 3. In the non-diet group three parents thought that food allergy might play a role in dermatitis but no parent in this group had noted any specific foods which exacerbated their child's dermatitis. However, over 50% of the parents in this group had heard or read of a possible association between dermatitis and diet. Asian parents in the non-diet group were far less likely to have heard or read about such an association (25% of Asians vs. 89% of whites, χ^2 $P<0{\cdot}02$). In the diet group all parents had heard of a link between diet and dermatitis and 46% thought that food allergy was the main cause of their child's dermatitis. In this group 35% of the parents thought that specific foods had exacerbated their child's dermatitis. However, a careful history revealed that nine of these 18 children developed skin changes immediately after contact with the food and were clearly experiencing contact urticaria. In the other nine cases the dermatitis was thought to deteriorate over the following 1–4 days. Most parents commented on the great difficulty of attributing blame to specific food items consumed in the few days previously.

The sources of information given by parents from which they had learnt about the possible role of diet are shown in Table 4. Only two general practitioners and one health visitor had discouraged the use of dietary changes. All the other community based health professionals had suggested that a trial of dietary alteration might be worthwhile. Health professionals mostly advised exclusion of cow's milk, eggs or all dairy and egg products, often suggesting substitution with goat or soya-based milks. However, very few of the parents could recall being given any specific advice on when benefit was to be expected, how long to continue the diet, and when and if to reintroduce the excluded foods. No one remembered being told of the possible hazards

TABLE 3. Parental views on cause(s) of eczema

Offered cause	Diet ($n=52$)	Non-diet ($n=21$)
No idea	12	10
Constitutional/Hereditary	8	6
'Definite' food allergy	24	0
'Possible' food allergy	0	3
Other allergy	9	2
Stress	3	1
Maternal diet (in pregnancy)	1	0
Other causes (e.g. weather, blood disorder)	3	2

TABLE 4. Sources of information regarding role of food allergy in eczema

Source	Number
Books/magazines/T.V./radio	37
Friends and neighbours	23
General practitioners	20
Hospital consultants	16
Health visitors/District nurses	13
National Eczema Society	9
Personal observation	5
Other:	
Clinical psychologist, herbalist, homeopath, schoolteacher	6

associated with re-challenge. None had been sent to see a dietitian. The only patients who had received dietetic supervision were those who had been started on complex exclusion diets in an Immunology clinic, all of whom were seen by a hospital dietitian. Fifty-four per cent of parents in the diet group had initiated dietary alteration before any form of professional advice had been sought.

There was no significant difference in birth weights, family history of atopy (χ^2 $P > 0{\cdot}1$), incidence of breast feeding (χ^2 $P > 0{\cdot}05$), personal history of additional atopic disease (χ^2 $P > 0{\cdot}5$) or in parental social class (χ^2 $P > 0{\cdot}5$) between the diet and non-diet groups.

Parental gradings of the severity of the dermatitis were slightly higher (i.e. more severe) than the doctor's in both diet and non-diet groups, and as judged by both parental and doctor grading, the group on diet had slightly more severe dermatitis. However, even in the diet group 46% were graded by the doctor as having very mild or mild dermatitis only (Grade 1 or 2), with

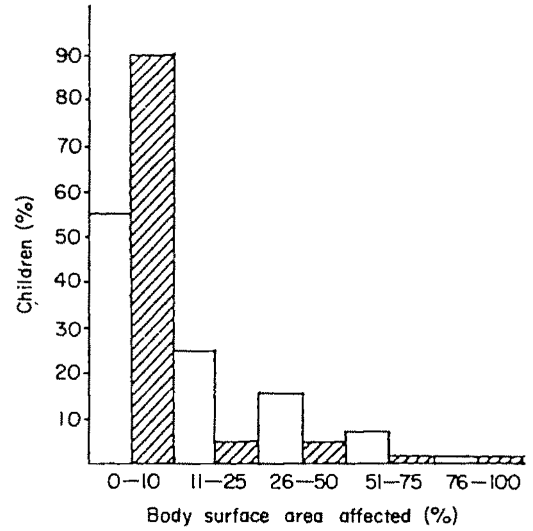

FIGURE 1. Table showing % of body area affected by eczema at time of study in diet and non-diet group. □, diet group; ▨, non-diet group.

54% having flexural disease occupying less than 10% of the body surface area (Fig. 1). Only 15% of the diet group and 5% of the non-diet group were considered to have severe dermatitis (doctor's Grade 4 or 5).

In both groups topical steroids were used appropriately in most cases. Only one case was encountered of obvious excess use (50 g of 50% Dermovate per week being applied to the body and face of a 6-year-old boy with mild to moderate dermatitis). However, in four instances there was a complete refusal to use even mild topical steroids in children with troublesome dermatitis (Grade 3 or over). This arose from misplaced parental anxiety about the side-effects of mild topical steroids. All these four children had been placed on special diets.

No significant differences in growth were demonstrated between the two groups of children (Mann–Whitney *U*-test; NS) and no case of failure to thrive or deficiency syndrome secondary to dietary manipulation was encountered in this series.

DISCUSSION

There have been few controlled studies performed to assess the role of dietary exclusion in childhood atopic dermatitis[2,15] and its efficacy has not been established. The most commonly quoted report[2] was a small study with a high drop-out rate in a highly selected group of patients. This study did suggest a benefit from exclusion of cow's milk and eggs. Even enthusiasts of such exclusion diets have pointed out that most dermatitis is mild and can be treated with simple topical applications of emollients and mild topical steroids.[8,12,14] It is also widely acknowledged that exclusion diets are difficult to achieve and need careful dietary advice and supervision.[1,3,9,13]

Our study has relied on the memory of past events by the children's parents. However, despite the inherent problems of this retrospective technique, it has clearly been demonstrated that dietary alteration is commonplace (71% of the children in this study) amongst children with atopic dermatitis seen in a dermatology clinic. Most of the children on special diets had only mild or moderate dermatitis, often limited to the flexures. Many of these children could have been managed adequately with reassurance, advice and appropriate simple topical therapy. Few parents felt that diets had improved the dermatitis, although it could be argued that this is a reflection of the uncontrolled and haphazard way in which most of the diets were instituted; for example cow's milk was frequently excluded but without exclusion of other cow's milk products. We have not encountered cases of failure to thrive in this study nor was significant growth retardation demonstrated in the diet treated group. This may reflect the small sample size and the fact that severely restricted diets formed only a small minority of the diet group. However, it is also possible that single measurements of growth might fail to detect earlier periods of growth delay if associated with subsequent catch up growth when a normal diet is resumed.

It is interesting to note that much of the advice on alteration of diets (especially cow's milk exclusion) came from general practitioners, health visitors and district nurses. It seems likely that many atopic children with mild dermatitis, who are never referred to hospital, are also being advised on special diets. The media and 'health industry' must also bear much of the responsibility for misguided and inaccurate opinions held by the public on this issue. Many parents firmly believed that goat's milk or soya-based milks were of proven benefit for childhood dermatitis.

In contrast to the dearth of evidence which exists to support the widespread use of diet in atopic dermatitis, several reports and reviews have been published drawing attention to the potential hazards of this practice.[5-11,16] These include failure to thrive[6] and vitamin and mineral deficiency,[11] as well as the potential serious hazards of feeding unmodified, and often unpasteurized goat's milk to small babies.[10] Furthermore, there may be adverse psychological effects of dietary restriction, particularly when children reach school age. The cost of inappropriate special diets (e.g. gluten free diets) can also be a significant burden to the family. The risk of re-introduction of excluded food items must be borne in mind.[5] Finally, some children clearly prefer dermatitis to dietary restriction.

The children who were on severe or potentially dangerous diets did not have severe dermatitis, and this parental infliction of misguided treatment on their children has been viewed by some to merge into the realms of Meadow's syndrome.[17] It is disturbing to note that both in our study and that of others[17,18] haphazard and unsupervised dietary restriction has often been initiated by doctors or other health professionals.

The large Asian population of Leicester enabled us to study ethnic variation in public understanding of the nature and management of atopic dermatitis. Fewer Asians tried dietary alteration as compared to Caucasian parents and this reflected the higher proportion of Asians who claimed no knowledge about the cause of dermatitis or its possible relation to diet. These findings are in keeping with those in other chronic conditions, such as diabetes[19] and renal failure, where patient education amongst Asians appears poor even in centres where Asians form a high proportion of the total population. Paradoxically this lack of awareness of a possible relationship between diet and dermatitis amongst many Asian families may have protected the children from erratic and unsupervised dietary alterations. There is an obvious need for better education about the nature of dermatitis amongst Asian families, but this needs to be promoted carefully so as to prevent the use of unsupervised diets in this group.

We conclude that dietary manipulation in a hospital clinic population of children with atopic dermatitis is extremely common, and is frequently applied to children with only mild dermatitis. Few parents felt that the diets had been helpful, although this may reflect the inadequate nature of the diets. Contrary to published advice, these diets are uncontrolled, unsupervised and are often continued indefinitely. This is a potentially serious health problem.

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nm, which is in the infra-red region of the electromagnetic spectrum. The energy at this wavelength is absorbed by water in tissues and can instantly vaporize the target tissue. At its focal point the laser beam can cut like a scalpel but in a haemostatic fashion. If the beam is defocused it can vapourize or simply coagulate tissue. The laser may thus be used to remove viral warts in a bloodless and self-sterilizing manner. It has also been suggested that, by 'sealing' nerve endings, CO_2 laser wounds are relatively painless.[8,9] We report the results of the use of CO_2 laser therapy for persistent cutaneous viral warts.

METHODS

During a 9 month period, 22 adult patients (13 female, nine male) with a median age of 29 years (range 16–64) were referred by colleagues for consideration of CO_2 laser treatment for their viral warts. Four patients have subsequently been lost to follow-up. Of the remaining 18, one patient was immunosuppressed following a renal transplant and the others were healthy. Twelve patients had plantar warts, five had warts on the fingers (mainly periungual) and one had warts on the fingers and palm. The median duration of the warts prior to laser treatment was 5 years (range 7 months–20 years).

Patients were accepted for laser therapy only as a last resort. The indications for laser therapy were in all cases the failure of other appropriate forms of treatment. Specific symptoms were pain or discomfort in the warts (16 patients), cosmetic embarrassment (11 patients) and orthopaedic complications due to abnormal weight bearing when walking (five patients). Treatments which had previously failed to clear the warts are shown in Figure 1. The average number of previous applications of cryotherapy in these patients was 14.

After obtaining informed consent, warts were treated under local anaesthesia (1% lignocaine with or without adrenaline) with the patient recumbent. The areas of wart tissue treated were solitary in eight cases and multiple in 10, up to a maximum of seven treated areas in one case. Nine of the patients had warts at distant sites which were not treated.

Warts were vapourized using a 20 W CO_2 laser (Xanar, Codman Limited, Maidenhead,

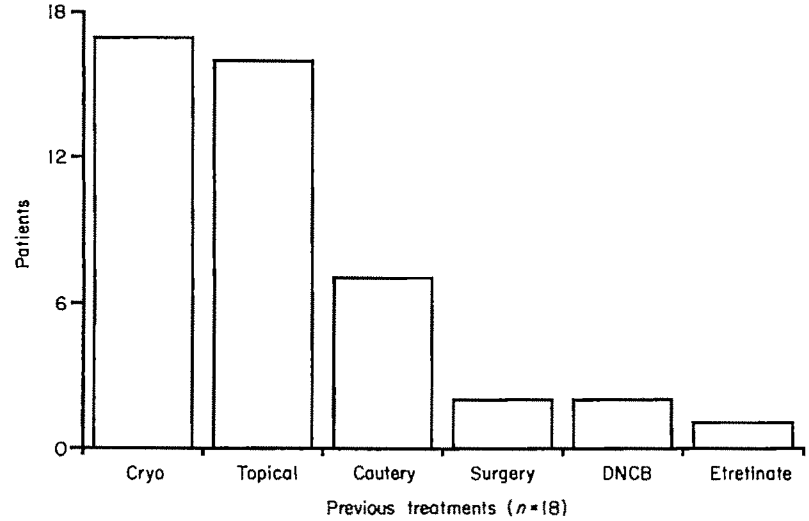

FIGURE 1. Treatments used on patients prior to CO_2 laser.

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Outcome of carbon dioxide laser therapy for persistent cutaneous viral warts

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SUMMARY

Twenty-two adults with persistent, symptomatic viral warts on the hands or feet which had failed to respond to conventional therapy were treated by carbon dioxide (CO_2) laser ablation under local anaesthesia. Four patients did not return for follow-up treatment. Of the remaining 18 patients, 12 had plantar warts, the other six had warts on the hands that were mainly peri-ungual. The median duration of the warts prior to laser treatment was 5 years. The areas treated were solitary in eight and multiple in 10 cases. After an average follow-up period of 10 months (minimum 6 months), all of the treated areas were clear of warts in 10 patients (56%), some had cleared in three, and all the treated warts had relapsed in the other five patients. Side-effects were commonly observed, especially post-operative pain, temporary loss of function of the treated part and scarring. The use of the CO_2 laser may clear otherwise resistant viral warts but a significant morbidity may occur with this method of treatment.

The majority of cutaneous viral warts will regress spontaneously within 3 months to 5 years of their onset.[1] There is evidence that both humoral and cell mediated immunity are involved in this process.[2] In the first 3 months, 20% of normal patients will experience resolution of their warts without treatment. This figure rises to at least 70% during the same time period if simple keratolytic paints are used, both for common warts and for deep plantar warts.[3] There remains a small group of otherwise normal patients whose warts persist for prolonged periods, sometimes many years. These may be a source of considerable discomfort and embarrassment to the patient, and various treatments have been employed to deal with them. These include destructive techniques, such as curettage, cautery and cryotherapy, immunostimulation (levamisole and dinitrochlorobenzene sensitization) and the use of intralesional agents such as bleomycin or interferon-α.[2] None of these treatments has yet proven ideal.

In the last few years there have been some encouraging reports from the United States concerning the use of the carbon dioxide (CO_2) laser for eradicating persistent cutaneous viral warts.[4–7] The CO_2 laser emits non-ionising, monochromatic radiation at wavelength 10,600

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Berkshire, U.K.) under direct vision guided by a Helium–Neon aiming beam. During the procedure, patients and staff wore protective goggles, and the immediate peri-operative area was protected by moist surgical drapes. The CO_2 laser beam was defocussed to achieve tissue vaporization, and set at a power output between 8 and 20 W depending on the thickness of the lesion to be treated. The laser beam was moved slowly over the wart in a series of passes. The smoke and vapour plume thus produced was carefully extracted using a vacuum system (Evaculase®, Codman). This is necessary to minimize hazard to patient and staff, as papilloma virus is present in the vapour of CO_2 laser-treated verrucae.[10] After each pass the resultant char was removed by gentle scrubbing with a cotton wool swab soaked in 3% hydrogen peroxide solution. Curettage was not used. This procedure was continued until all visible signs of wart tissue had been removed and the dermal rete ridge pattern was restored (Fig. 2). The assesment of wart removal was facilitated by the fact that viral wart tissue bubbles and spatters under the laser beam in a manner quite different from the melting and contraction demonstrated by the surrounding dermal connective tissue. An average of four passes of the laser was required to remove the warts. The first pass of the laser included a zone approximately 5 mm wide beyond the visible edge of the wart, as papilloma virus has been demonstrated in apparently normal epidermis surrounding viral warts.[11]

Post-operatively, the treated areas were cleaned twice daily with 3% hydrogen peroxide solution and covered with a thin layer of mupirocin (Bactroban®) under non-adherent dressing until re-epithelialization had taken place. Patients were followed-up at approximately 2-weekly intervals for 4–6 weeks. Laser treatment was repeated in one patient only after 3 weeks. After a minimum of 6 months all patients were sent a detailed questionnaire and invited to attend for clinical examination.

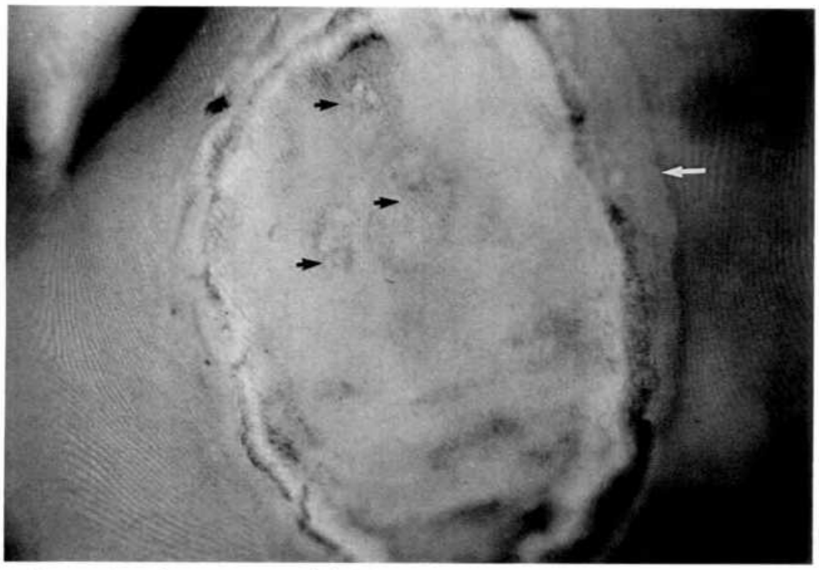

FIGURE 2. Appearance after two passes of the laser and removal of charred tissue. The dermal rete ridge pattern is largely restored, but three islands of residual wart remain (black arrows). The peripheral skin treated by one pass of the laser is demonstrated (white arrow). Note the absence of bleeding.

RESULTS

Four patients were lost to follow-up. The remaining 18 (11 female) returned the questionnaire, and all but two attended for re-examination after an average of 10 months (range 6–14 months) post-operatively.

Cure rate

At follow-up five patients with plantar warts were all clear, in two cases some, but not all, treated areas were clear and in the other five patients all treated areas showed recurrence of wart tissue. The clearance rate for hand warts was higher, five out of six patients showing no recurrence, and in the other patient three of the four treated areas were still clear of wart. The overall cure rate was thus 56% (10 out of 18 patients). Untreated warts at distant sites were not seen to resolve even after the successful eradication of warts at treated sites.

Scarring

Scarring in treated areas was apparent in 11 patients. In eight cases the scars were tender on digital pressure or when walking. Both atrophic (Fig. 3) and hypertrophic (Fig. 4) scars were seen. The development of scarring was not influenced by the duration or site of the warts, nor by the number of passes of the laser used.

Pain

This was experienced after laser treatment by all patients. In four cases it was described as mild to moderate in severity. However, 14 patients reported the post-operative pain to be severe, lasting a median duration of 3 weeks. One patient (Fig. 3) was still experiencing severe

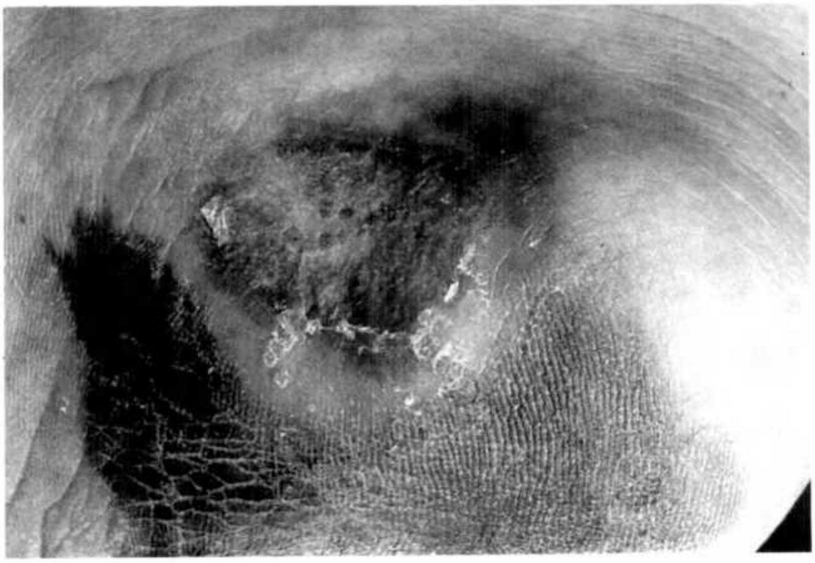

FIGURE 3. An atrophic, tender scar on the side of the heel after laser treatment. Small recurrences of wart can be seen within the scarred area.

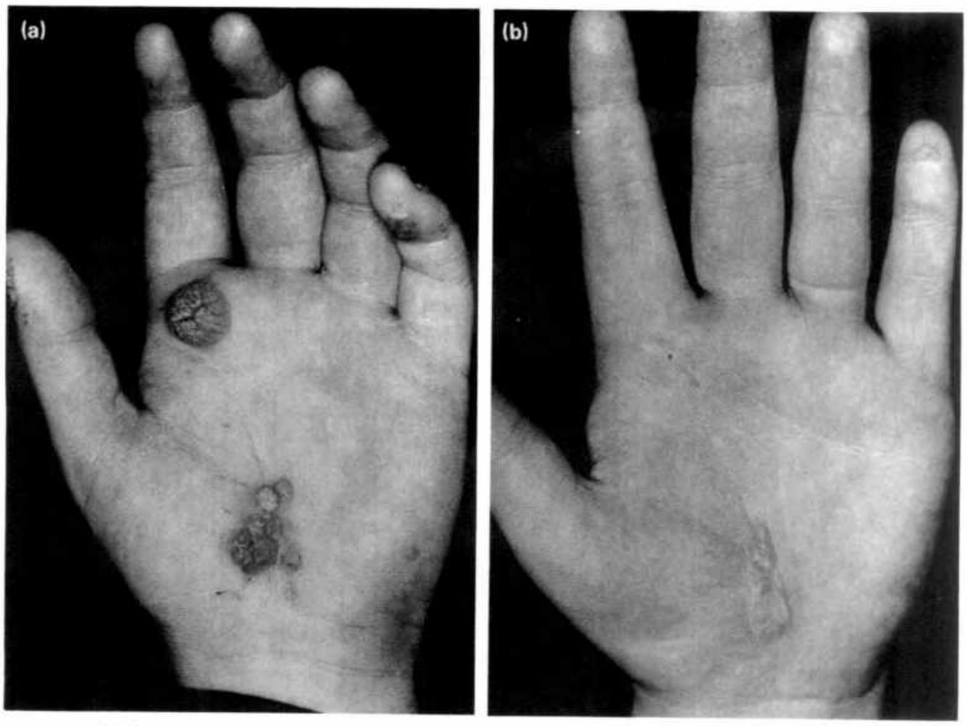

FIGURE 4. (a) Appearance of unsightly and painful hand warts prior to laser treatment. (b) After 6 months the warts are gone but there is hypertrophic scarring at the treatment sites. The patient was pleased with the outcome.

discomfort on weight bearing 9 months post-operatively, and this had prevented her returning to work as a hairdresser. Only one patient did not need to take any post-operative analgesia. Analgesics ranging in potency from paracetamol to an opiate (in one case) were required by the other 17 patients for an average of 2 weeks. In many cases the pain was only experienced on attempting to bear weight on a treated area.

Infection

Although the wounds were allowed to heal by secondary intention and the mean time to full re-epithelialization of all treated areas was 9 weeks, there was a low rate of infection in these patients. Only four patients gave a history suggesting post-operative infection. This was probably due to the sterilizing action of the laser and the routine use of topical mupirocin.

Bleeding

This was not a significant problem and although reported by 14 patients, was usually trivial and occurred at times of dressing changes.

Disturbance of function

Of the 12 patients who had plantar warts treated, 10 experienced some difficulty with weight bearing afterwards. In six cases, normal weight bearing on walking was not possible for periods ranging between 2 weeks and 9 months in one case. Four patients were unable to walk at all for an average of 13 days (3–35 days). Of the five patients with pre-existing orthopaedic difficulties from their plantar warts, three were unchanged, one had cleared and one was worse after laser treatment. At follow-up, four of the five patients with hand warts had full restoration of hand function, although this had been a slow process and there was sensitivity of the treated areas on the fingers for several weeks in three patients. One patient had a persistent contracture of the treated finger and associated poor grip. Seven (39%) of the patients treated had lost time from work (median 7 days) following laser treatment.

Patients' assessment

Opinion was approximately equally divided amongst the patients regarding the overall efficacy of the laser therapy. Eight patients were satisfied that the treatment had improved their eventual clinical status despite the acute morbidity, whilst nine patients felt that they were worse off after the treatment. One patient was undecided.

DISCUSSION

In this study, using a CO_2 laser we have achieved a 56% rate of cure of hand and foot warts that had failed to respond to other treatments. However, there were a disturbing number of side-effects, especially scarring (61%), post-operative pain and disturbance of function. Previous workers in this field have produced more optimistic findings,[4–7] with cure rates for plantar warts ranging from 55[7] to 95%[4] and 82% for hand warts.[6] The incidence and severity of post-operative pain have been quoted as 'minimal'[4,7] or mild in 30% of cases.[6] Likewise, scarring has only been mentioned in one paper, occurring in half of the cases treated for finger warts.[5] The slow rate of healing seen in our study is a recognized feature of laser wounds,[5,12] and scarring after cutaneous CO_2 laser surgery (all indications) has been experienced by 64% of physicians in a recent survey.[13]

Our results are not as encouraging as those previously reported. Differences in patient selection, operative technique and after-care may all have contributed to this. There is no doubt that the patients included in this study represented a highly selected group with most resistant warts. The warts had been present for a median period of 5 years, when the rate of spontaneous remission must be low. Previous papers give little information on this point, except for Borovoy's study[7] where most of the treated lesions had been present for one year or less. The cumulative effect of numerous previous treatments in our patients (Fig. 1) may have mitigated against a good result in some cases. Secondly, in this study the average amount of laser exposure time (10–15 min; four passes at 8–20 W) was greater than that documented in other reports. Perhaps this is an indication of the greater wart size treated in our study. However, longer exposures to relatively low power densities (laser beam defocused) is known to lead to greater spread of non-specific thermal injury into surrounding tissues with a consequent increase in the risk of scarring. Newer developments such as the superpulsed laser may help to overcome this problem.[14] Curettage, used either before [7] or during[6] the laser procedure will have debulked the warts, thereby reducing the total exposure required in these studies. The use of the operating microscope[4,5,7] and of acetic acid,[7] may both help in the delineation of the wart tissue, thereby minimizing inadvertent laser damage to surrounding normal tissue. Finally, because of the slow healing of laser wounds, the choice and frequency of dressing may have a significant bearing on the rate of healing and post-operative pain.

This study has confirmed the ability of the CO_2 laser to eradicate otherwise resistant cutaneous viral warts. However, critical appraisal of our patients at follow-up has demonstrated less than perfect results as measured by several criteria. Having stated these publicly, we were interested to hear that others have experienced similar disappointing cure rates and scarring. It is necessary for laser users to accurately define the patient groups for whom such treatment is indicated. This will require a long-term, parallel study comparing laser treatment with modalities such as bleomycin or interferon, taking into account recurrence rates, complications and costs.[15]

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A double-blind comparison of acitretin and etretinate in combination with bath PUVA in the treatment of extensive psoriasis

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SUMMARY

Thirty-four patients with extensive psoriasis were treated in a double-blind parallel fashion with either acitretin plus bath PUVA (trimethylpsoralen bath + UVA) or etretinate plus bath PUVA. Each group consisted of 17 patients. The dose of retinoid was 40 mg/day during the 2-week monotherapy phase and subsequently 20 mg/day during the combination treatment. Bath PUVA was given three times a week starting with a UVA dose of 0·06 J/cm². Remission (> 90% improvement) was achieved in all patients in 6–10 weeks. There were no significant differences in clinical response between the two groups; the mean ± SD PASI score (psoriasis area and severity index) before treatment was 22·6 ± 7·1 in the acitretin-PUVA group and 19·4 ± 7·8 in the etretinate-PUVA group. The corresponding figures after treatment were 0·6 ± 0·6 and 1·0 ± 0·5, respectively. Side-effects related to retinoid treatment were frequent in both groups but they were usually mild and well-tolerated. There was only one case of diffuse alopecia after 8 weeks in the etretinate-PUVA group. Scaling of the palms and soles was seen in six patients in the acitretin-group but only in two patients in the etretinate-group. Triglycerides were elevated in about half of the patients in both groups. The present study shows that acitretin is as effective as etretinate in the combination with bath PUVA.

Several studies have shown that the combination of etretinate and systemic PUVA[1,2] or bath PUVA[3,4] is more effective than either treatment given alone. These combinations have proved useful in psoriatic patients not responding sufficiently to conventional therapies.

Etretinate has the disadvantage of a long elimination time. Its main acid derivative, acitretin, with a much shorter elimination half-life of 2–4 days[5] has recently proved to be at least as effective as etretinate in the treatment of psoriasis.[6–8] However, it seems to induce more side-effects, especially palmoplantar scaling and alopecia, than the parent compound.[6–7] In

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combination treatment, retinoids can be given in lower doses and they are better tolerated. In the present study we examined the effect of low dose acitretin in combination with bath PUVA and compared its efficacy and tolerability with the combination of etretinate and bath PUVA.

In Finland, bath PUVA (psoralen bath + UVA) is more often used than systemic PUVA and is considered more safe. Systemic absorption of the psoralen through the skin is minimal[9] and no nausea is induced. Also there is no accumulation of the psoralen in the eyes, especially in the lens, so protective glasses are not required following the treatment and there is less risk of cataracts. Either trimethylpsoralen[9-10] or 8-methoxypsoralen[10-11] can be used for bath PUVA.

METHODS

This study was performed in a double-blind parallel fashion. The psoriatic patients were randomly allocated to two different treatment groups: acitretin + bath PUVA or etretinate + bath PUVA. During the first 2 weeks the patients received retinoid alone (monotherapy phase), then the bath PUVA was started and the patients received combination treatment until clearing or up to 12 weeks.

Thirty-four patients (32 males, two females) with widespread plaque-type psoriasis were recruited for this study. Each treatment group consisted of 17 patients. The mean age of the patients in the acitretin-PUVA group was 41·9 ± 13·1 (mean ± SD) years and in the etretinate-PUVA group 36·1 ± 8·3 years. The mean weight of the patients in the acitretin-PUVA group was 84·2 ± 18·7 kg and in the etretinate-PUVA group 77·9 ± 10·2 kg. Capsules of 10 mg acitretin or 10 mg etretinate identical in size and colour were used. The daily dose was 40 mg/day during the first 2 weeks (monotherapy phase) and 20 mg/day during the combination therapy phase. The tablets were taken once a day during the main meal.

Trimelthylpsoralen solution (Tripsor®; Orion Pharmaceuticals, Espoo, Finland) was used, and one bottle containing 50 mg in 100 ml of alcohol was poured into 150 litres of water in a bathtub. The bathing time was 10 min. Lesions of the face were treated during bathing with 0·01% trimethylpsoralen cream (Tripsor®, Orion Pharmaceuticals). After bathing, the skin of the patient was gently dried and any cream wiped off the lesions with a paper towel. After that the patient was exposed to UVA radiation using the PUVA-22 UVA cabin (Airam Ltd, Finland). The radiation intensity of this cabin at the treatment distance is 10 mW/cm^2. Treatment was given 3 times a week. The starting dose was 0·06 J/cm^2. The dose was increased after every three exposures to 0·15, 0·25 and 0·4 J/cm^2 in all patients, and in patients who were not cleared at this phase, the dose was increased in the same manner to 0·55, 0·7 and even to 0·9 J/cm^2 in some patients in order to achieve the optimal clearing. The patients were allowed to use emollients on the skin and on the scalp. No other treatments were allowed.

The patients were examined at 2-week intervals. At each visit the intensity of erythema, desquamation, infiltration and pustulation were rated according to the following scale: 3 = severe, 2 = moderate, 1 = mild, 0 = absent. In addition, the percentage of involved body surface area was recorded. The severity of disease was evaluated by means of the 'Psoriasis Area and Severity Index' (PASI score) based on the above parameters.[7] The main criterion of efficacy was the improvement of the PASI score. Remission was designated as more than 90% improvement of the PASI score.

Clinical adverse reactions were recorded at each visit. The type and intensity were rated as mild, moderate or severe. Complete laboratory examinations (blood count, serum biochemistry and urinalyses) were performed at entry and at weeks 4 and 12 or at the end of the study. Transaminases and blood lipids were additionally checked at weeks 2 and 8.

For statistical analysis of the homogeneity of the two treatment groups, Fisher's exact test (two-tailed) was used with respect to sex, whereas analysis of variance was applied for age and weight. The Wilcoxon 2-sample test was used to compare PASI scores at baseline. The percent PASI score improvement, the duration of treatment until remission, the number of PUVA exposures and the total amount of UVA energy were considered as efficacy parameters. The null hypothesis of no difference between the treatment group was tested by means of the Wilcoxon 2-sample test.

RESULTS

Both treatment regimens proved effective in the treatment of psoriasis. Remission (more than 90% improvement of the PASI score) was achieved in all patients in 6–10 weeks. The mean ± SD PASI score of the whole patient population before treatment was 20·9 ± 7·5 (range 9–35·2) and that at the end of treatment was 0·8 ± 0·5 (range 0–1·7). Slight residual lesions were seen especially on the legs and scalp.

The mean PASI score in the acitretin group before treatment was 22·6 ± 7·1 (range 9·0–34·2) and that in the etretinate group was 19·4 ± 7·8 (range 12·0–35·2). The corresponding figures at the end of treatment were 0·6 ± 0·6 (range 0–1·7) and 1·0 ± 0·5 (range 0·2–1·7) respectively. Differences were not statistically significant. However, six patients were completely free of lesions after treatment i.e. they did not have even small erythematous patches on their skin (PASI score 0); all of these patients belonged to the acitretin-PUVA group.

The decline of the PASI scores during treatment (Fig. 1) was roughly parallel in both treatment groups. There were no major differences in responses to the treatments in either phase of the study. Figure 2 shows the body surface involvement with psoriasis. There was a slight extension in both groups in the mean area involved during the initial 2 weeks. At the same

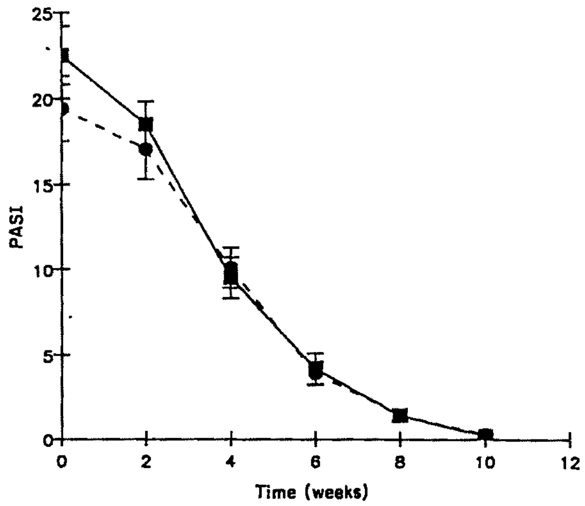

FIGURE 1. Decline of the PASI scores during treatment (mean ± SEM). —■—, acitretin; --●-- etretinate.

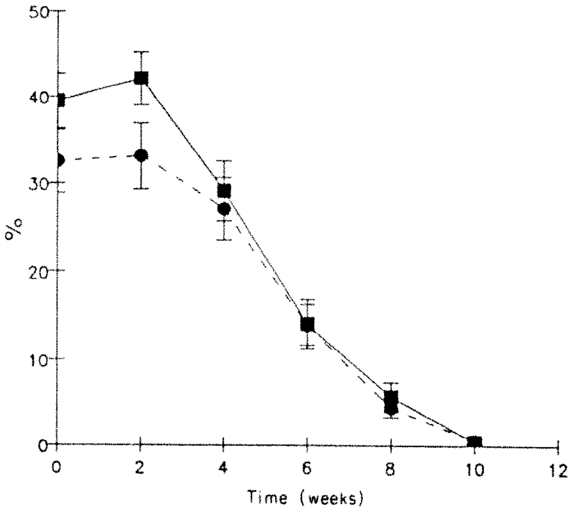

FIGURE 2. Reduction in the extent of psoriasis (percentage of involvement; mean ± SEM) during treatment. There is a slight extension of the mean area involved in both groups after 2 weeks (statistically not significant). —■—, acitretin; - -●- -, etretinate.

time the thickness of the horny layer of the lesions was reduced in all patients, and in some patients the lesions looked somewhat thinner with central clearing. Sometimes the lesions looked more red. After starting the simultaneous PUVA therapy the area involved declined roughly in parallel in both groups as shown in Figure 2. The time needed for clearing showed the same distribution in both groups. Four of the patients were in remission after 6 weeks, seven patients after 8 weeks and six patients after 10 weeks. Table 1 shows the mean duration of the treatment, the number of PUVA exposures and the total UVA dose delivered. The null hypothesis of no difference between the treatment groups could not be rejected at a 5% significance level for any of these parameters.

The patients were examined 12 months after stopping treatment and two patients in each group were still in remission. The mean remission time in the acitretin group was 6·8 ± 3·4 months (range 2–12 months) and in the etretinate group 7·4 ± 3·1 months (range 2–12 months).

Side-effects, mostly related to retinoid treatment were frequent in both groups. However, they were usually graded as mild to moderate in intensity and in all cases they were well-tolerated. Dryness of the mucous membranes (at least lips) was seen in all patients. Dryness of the skin was seen in seven patients (41%) in the acitretin group and in five patients (29%) in the

TABLE 1. Duration of treatment until remission (> 90% clearing), number of UVA exposures and amount of UVA energy (mean ± SD)

Group	Duration of treatment (days)	Number of PUVA exposures	Total of UVA J/cm^2
Acitretin-PUVA	57·2 ± 11·3	17·3 ± 4·0	6·1 ± 2·9
Etretinate-PUVA	56·9 ± 11·1	17·5 ± 3·9	6·2 ± 2·7

etretinate group. Mild to moderate pruritus was experienced by 11 patients (65%) in the acitretin PUVA group and by eight (47%) patients in the etretinate PUVA group. Scaling of the palms and soles was seen in six patients (35%) in the acitretin group and in two patients (12%) in the etretinate group; the difference was not statistically significant. One case of mild alopecia was seen in the etretinate group after 8 weeks. A case of paronychia and one patient with headaches were seen in the acitretin group, and single cases of nausea, tiredness, vertigo and arthralgia in the etretinate group. PUVA treatment caused mild to moderate erythema of the skin in three patients who were all in the acitretin group.

Elevation of at least one of the transaminases was found in five (29%) patients of the acitretin group with normal pretreatment values. The elevations were mild, only to 64 IU/l (upper normal limit 40 IU/l). In the etretinate group, four patients (24%) with normal pretreatment values showed mild elevations (up to 68 IU/l) during treatment. In the acitretin group, serum triglycerides were elevated (> 1·7 mmol/l) in eight patients (47%) with normal pretreatment values. The highest level was 5·1 mmol/l. The pretreatment level was elevated in four patients. In the etretinate group, nine patients (53%) with normal pretreatment values showed elevation during treatment with the maximum of 4·6 mmol/l, and four patients had an elevated pretreatment level. A raised level of serum cholesterol was not so frequent and in the acitretin groups in only one patient with a normal pretreatment level (< 7·0 mmol/l) did it become elevated. In the etretinate group, three patients had values up to 8·5 mmol/l. Elevated pretreatment levels were seen in three patients in the acitretin group and in two patients in the etretinate group.

DISCUSSION

The combination of either acitretin or etretinate plus bath PUVA was effective in the treatment of extensive psoriasis, remissions being achieved in all patients. However, the mean time taken to achieve remission was about 8 weeks. Higher dosage of the retinoids and/or UVA might have offered a more rapid clearing, but the dosage used in this study was well-tolerated. Clinically there was only one case of diffuse alopecia after 8 weeks and all other patients experienced only minor discomfort, although laboratory examinations revealed elevations, especially of triglycerides, in about the half of the patients.

The initial retinoid dose of 40 mg/day during the monotherapy phase seemed to induce a sufficient desquamative effect in most patients. At the same time the lesions of some patients showed spreading which was reflected in the extension of the mean surface area involved (Fig. 2). The mean PASI score declined because of reduced scaling and thickness of the lesions after monotherapy. At this phase the psoriatic lesions were most responsive to PUVA therapy with a low dosage of UVA exposure starting with 0·06 J/cm^2 and given three times a week. For bath PUVA given alone the dose needed is usually 1/3–1/2 more than in this study. A previous comparative study has shown that the cumulative UVA dose needed is reduced by about 50% when the combination treatment is used.[4] Higher doses might have given more rapid results in some cases in our study but skin erythema would have been more of a problem. In the present study, there were only three cases of mild to moderate erythema.

The low doses of PUVA needed in bath PUVA, and especially in the combination of retinoid and bath PUVA, do not necessarily reduce the long-term photodamage of the skin since the phototoxicity reaction in the skin is about the same with systemic PUVA in which much higher doses of UVA are used. During retinoid pretreatment, the thickness of the horny layer is reduced and this increases the penetration of both psoralen from the bath and UVA radiation into the lesions with subsequent combination therapy of retinoid and bath PUVA. A lower dose of UVA is needed to bring about a therapeutic effect in the lesions and thus the surrounding uninvolved skin will receive a lower dose. This is likely to reduce the risk of long-term photodamage in patients who need repeated treatment courses.

Bath PUVA is a very effective and safe method of treating psoriasis. The combination of a retinoid and bath PUVA allows a reduction in the dose of UVA which in turn reduces the photodamage of the uninvolved skin. There are side-effects from the retinoids but usually they are not a major problem at these low doses and with short lengths of treatment. On the basis of this study, acitretin is as effective as etretinate. Acitretin may in future replace etretinate in combination treatments because of its shorter elimination time and the shorter period for potential teratogenicity.

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Multinucleate cell angiohistiocytoma: a clinicopathological, immunohistochemical and ultrastructural study

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SUMMARY

The term 'multinucleate cell angiohistiocytoma' was first introduced by Smith and Wilson Jones in 1985. We report the clinicopathological, immunohistological and ultrastructural findings observed in two patients. Multinucleate cell angiohistiocytoma occurs mainly in middle-aged women and is usually located at acral sites, particularly the distal extremities. Grouped, brown-red, slightly elevated, asymptomatic papules slowly develop over several months until further growth ceases. There is no evidence of systemic disease. Histologically, the dermis shows numerous well developed capillaries with prominent endothelia, large bizarre basophilic and often multinucleate cells with a sparse lymphohistiocytic infiltrate. The immunohistological and ultrastructural findings suggest a fibroblastic differentiation of the large multinucleate cells.

Multinucleate cell angiohistiocytoma is a skin disorder, first reported by Smith and Wilson Jones.[1,2] The grouped, brown-red skin papules are histologically characterized by an angiomatous component associated with bizarre multinucleate giant cells. In the present study we report two cases of this disorder. The clinical, histological, immunohistological and ultrastructural findings suggest that multinucleate cell angiohistiocytoma is a distinct entity.

CASE REPORTS

Case 1

A 39-year-old female patient presented with a 3-year-history of skin lesions on the face. The lesions developed over several months and finally stopped growing but showed no tendency to resolve. On the lower lids of both eyes there were a number of grouped, non-tender and slightly elevated, papules (Fig. 1), which were brown-red in colour and 4–8 mm in size. These papules

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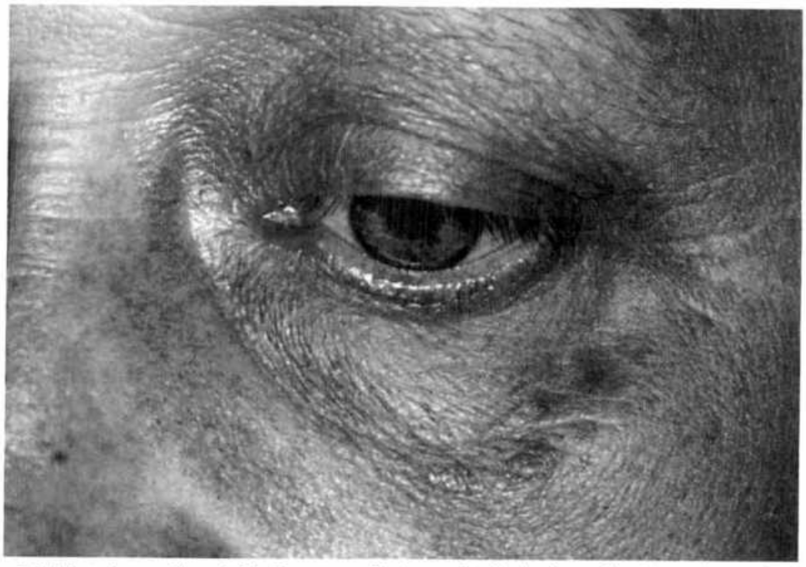

FIGURE 1. Multinucleate cell angiohistiocytoma. Patient 1. Indistinctly outlined papules on the lower left eyelid.

were indistinctly outlined with a smooth surface. Laboratory investigations including, red and white blood cell count, blood glucose, liver and renal function tests were normal. There was no evidence of any systemic disease.

Case 2

A 57-year-old female patient had had slowly progressive skin lesions over a period of 4 years.

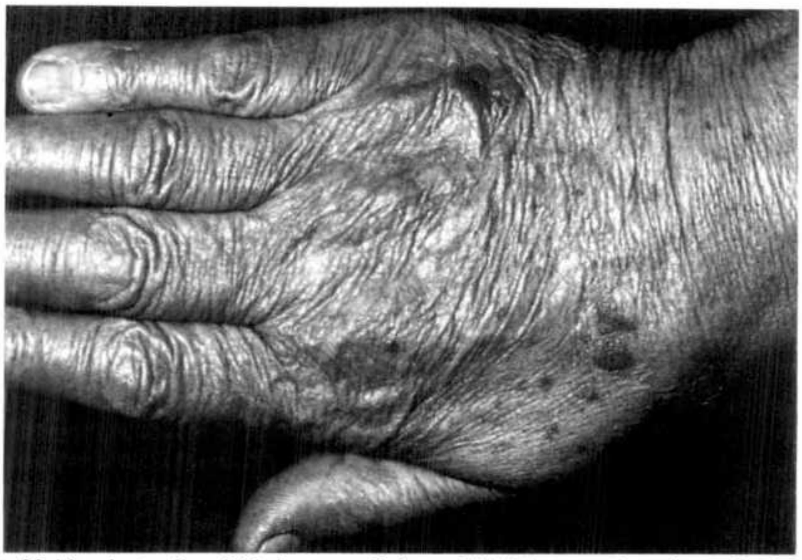

FIGURE 2. Multinucleate cell angiohistiocytoma. Patient 2. Multiple grouped papules on the dorsal aspect of the right hand.

Asymptomatic dark red, slightly pigmented dome-shaped papules with a diameter of 5–10 mm and a smooth surface were arranged in groups on the dorsum of the right hand (Fig. 2) and on both knees. Laboratory findings including red and white blood cell count, blood glucose test, liver function tests and renal function tests were again normal.

METHODS

Specimens

From each patient, two punch biopsies were fixed in phosphate buffered formaline and routinely embedded in paraffin. One punch biopsy from each patient was snap frozen in liquid nitrogen and stored at −70°C. From Case two, one part of a punch biopsy was used for electron microscopy.

Histology

5 μm paraffin-embedded sections were cut and stained with hematoxylin and eosin, van Gieson, orcein, PAS and alcian blue.[3]

Immunohistology

Mouse monoclonal antibodies and polyclonal rabbit antisera were used as first reagents in a three-step or two-step immunoperoxidase procedure as previously described.[4] The antibodies and antisera used are listed in Table 1. Non-neoplastic tonsils and normal skin served as positive controls. For negative controls, the primary antibodies were omitted or replaced with normal serum of the same species.

TABLE 1. Multinucleate cell angiohistiocytoma. Immunohistological results

Antibody	Source	Specificity	Results
CD 3*	BD	pan-T-cells	positive cells in the lympho-histiocytic infiltrate
CD 4*	BD	Helper/inducer cells	positive cells in the lympho-histiocytic infiltrate
CD 8*	BD	Suppressor/cytotoxic cells	few positive cells in the lympho-histiocytic infiltrate
CD 19*	BD	pan-B-cells	negative
CD 1*	Ortho	Langerhans cells/indeterminate cells	negative
OKM 1*	Ortho	monocytes	few positive cells in the lympho-histiocytic infiltrate
OKM 5*	Ortho	monocytes	few positive cells in the lympho-histiocytic infiltrate
MAC 387‡	Dako	monocytes	negative
BMA 120†	Beh	endothelial cells	positive in the vascular component
F VIII-RA†	Dako	endothelial cells	positive in the vascular component; weakly positive in some multinucleate cells in frozen sections
F XIII‡	Beh	some fibroblasts	negative
Desmin‡	Dako	muscle cells	negative
Vimentin‡	Dako	mesenchymal cells	positive in the multinucleate cells
Keratin‡	Dako	epithelial cells	negative
Lysozyme‡	Dako	histiocytes	negative

* Performed on frozen sections only.

† Performed on frozen sections and paraffin-embedded sections.

‡ Performed on paraffin-embedded sections only.

BD = Becton Dickinson, U.S.A.; Ortho = Ortho Pharmaceutical Corporation, U.S.A.; Beh = Behring, F.R.G.; Dako = Dakopatts, Denmark.

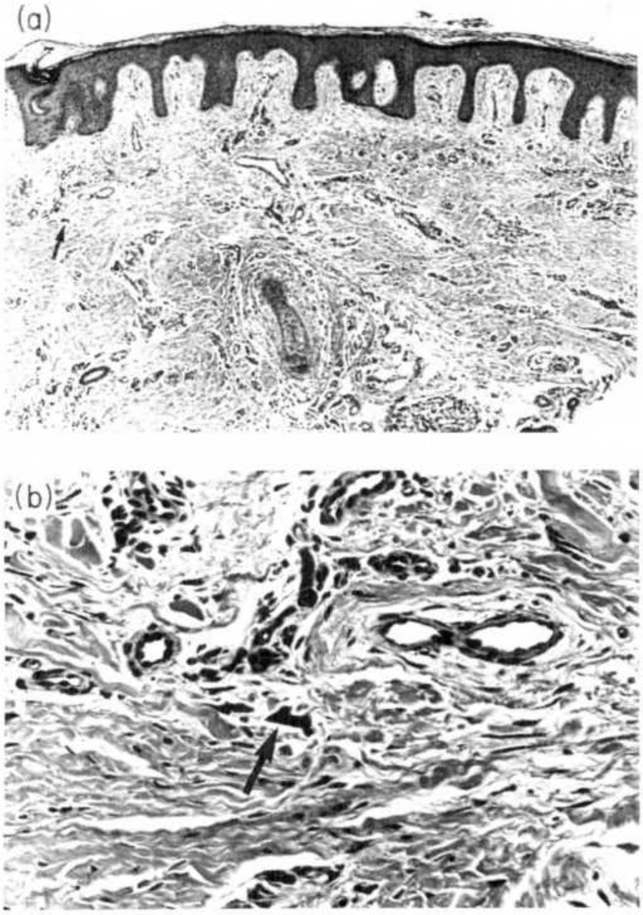

FIGURE 3. Multinucleate cell angiohistiocytoma. Histology. (a) Numerous dilated blood-vessels and some large, prominent cells (arrow; H&E, original × 50). (b) Dilated vascular spaces and numerous small capillaries with prominent endothelial nuclei. Large, bizarre, strongly basophilic cells are distributed in the surrounding dermal tissue (arrow; H&E, original × 250).

Electron microscopy

Small specimens measuring about 1 mm^3 were fixed in 3% glutaraldehyde (cocadylate buffer, pH 7·3), postfixed in 2% osmium tetroxide and subsequently embedded in Epon 812. Ultrathin sections were prepared using a Reichert Ultracut (Reichert, Austria). The sections were counterstained with lead hydroxide and uranyl actetate solution and examined with an electron microscope Zeiss EM 9 S 2 (Zeiss, F.R.G.).

RESULTS

Histology

All specimens revealed similar histologic features (Fig. 3). The epidermis was unchanged or slightly acanthotic. In the papillary dermis and particularly in the mid-dermis there was a marked increase of blood-vessels that were round or elongated. Some of the vessels showed a thin but distinct smooth muscle layer and others were well defined capillaries embedded in loose connective tissue. In most of the vessels, the endothelial cells had prominent nuclei that appeared to project into the lumen. Occasionally around the blood-vessels there was a sparse lymphohistiocytic infiltrate.

In the mid-dermis, large irregularly shaped cells were found. These showed either one large nucleus with finely dispersed chromatin, or multiple, occasionally hyperchromatic nuclei. The cytoplasm was strongly basophilic and displayed a dendritic or a bipolar outline. The cells were embedded in normal dermal connective tissue.

Orcein and van Gieson stains revealed delicate elastic fibres and fine collagen fibres adjacent to the prominent dermal vessels, and a normal dermal architecture in the neighbourhood of the multinucleate cells. PAS positive and alcian blue positive material was found near the dermal vessels, but not in the dermal tissue surrounding the multinucleate cells.

Immunohistology

The vascular component of the lesions was clearly labelled with factor VIII-related antigen and BMA 120 (Fig. 4). Multinucleate cells were negative for BMA 120, but were weakly positive for

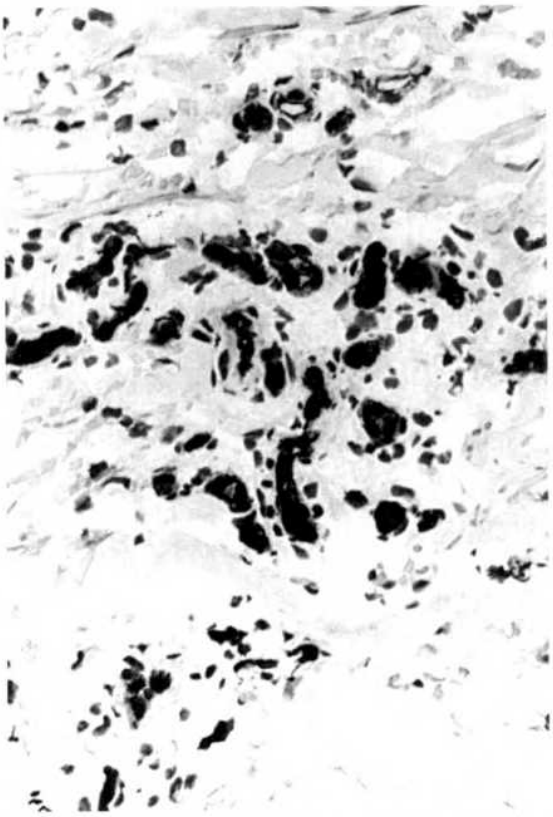

FIGURE 4. Multinucleate cell angiohistiocytoma. Immunohistochemical demonstration of vascular endothelia. There is a cluster of small vascular profiles with a positively stained endothelial lining. (BMA 120 monoclonal antibody, three-step immunoperoxidase method, original × 250).

factor VIII related antigen. Otherwise, the multinucleate cells were only reactive with vimentin. Monocyte–histiocyte markers were negative. The small foci of lymphohistiocytic infiltrate showed a predominance of T-helper-inducer cells associated with a few monocytes–histiocytes.

Electron microscopy

The blood-vessels were mainly well developed capillaries with a continuous endothelial tube and occasional pericytes. The endothelial cells showed prominent nuclei and numerous cytoplasmic processes at the luminal surface. Additionally, metarterioles displaying an incomplete layer of irregularly spaced smooth muscle cells were present. Among the large bizarre cells, five cell types could be discerned. (i) Typical fibroblasts with an elliptical nucleus and fine elongated cytoplasmic processes. (ii) Large, bi- or tripolar cells with two or three nuclear profiles. The nuclei showed a band of fine-textured material adjacent to the inner membrane of the nuclear envelope. The cytoplasm displayed a prominent rough endoplasmic reticulum (Fig. 5). (iii) Large elongated cells with numerous nuclei at distant cell poles. The nuclei resembled those of fibroblasts with respect to the fine chromatin architecture and the abundance of euchromatin. (iv) Multinucleate giant cells, whose nuclei were dispersed throughout the whole cell body (Fig. 6). There were no nuclear bridges. (v) Multinucleate giant cells, which were even larger than the previous ones. In this cell type, up to 15 nuclei were located mainly at the periphery of the cell. The cell types (iii) to (v) showed cystic dilations of the endoplasmic reticulum and numerous small cytoplasmic processes at the surface. The collagen

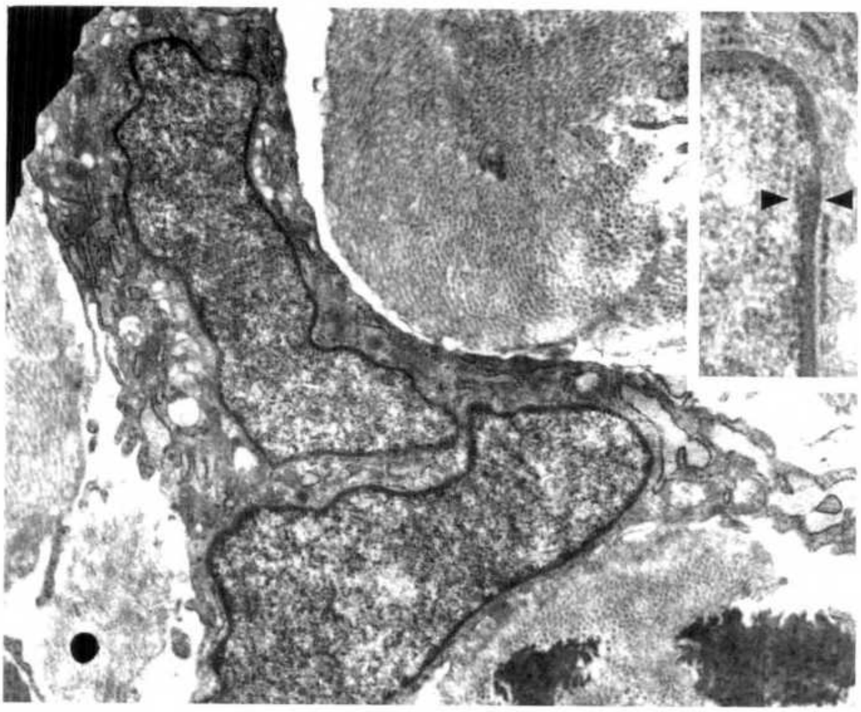

FIGURE 5. Multinucleate cell angiohistiocytoma. Electron microscopy. Large fibroblast with two distinct nuclear profiles without nuclear bridges, a zonula nucleum limitans (inset; arrows) and a prominent rough endoplasmic reticulum. Magnification, Original × 11,270 (inset Original × 54,810).

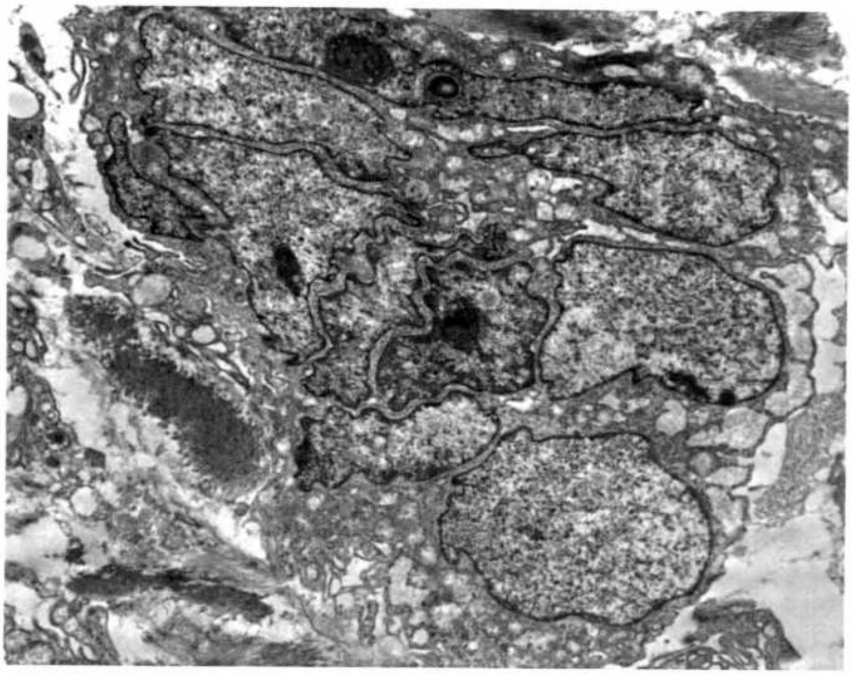

FIGURE 6. Multinucleate cell angiohistiocytoma. Electron microscopy. Multinucleate giant cell with 10 nuclear profiles distributed throughout the whole cytoplasm. The cytoplasm shows a partially dilated rough endoplasmic reticulum. Magnification, original × 5710.

fibrils did not show any structural abnormalities. Around the blood-vessels, delicate bundles of collagen fibrils were evident, whereas the multinucleate cells were embedded in a coarsely textured collagenous network. Elastic fibres were present at normal amounts.

DISCUSSION

Both of our patients had the typical clinical features of multinucleate cell angiohistiocytoma as reported by Smith and Wilson Jones[1,2] occurring in middle-aged women with slowly progressive skin lesions without any tendency to resolve. They were localized on the extremities and face and were reddish-brown, slightly elevated asymptomatic papules. The differential diagnosis included lichen planus, Kaposi's sarcoma, histiocytoma and granuloma annulare.[1,2] On histology multinucleate cell angiohistiocytoma was characterized by prominent dermal vessels, peculiar multinucleate cells and occasional discrete foci of a lymphohistiocytic infiltrate. The most striking feature are the large, bizarre, often multinucleated cells with a strongly basophilic cytoplasm, which are situated within dense dermal connective tissue without any apparent association with the vascular component or the inflammatory infiltrate.

The fibrous papule of the nose may resemble a multinucleate cell angiohistiocytoma[5,6] but his usually occurs as a single lesion. The dermal collagen in this lesion shows a vertical orientation, in contrast to the normal more or less horizontal orientation in the multinucleate cell angiohistiocytoma. Additionally, the vascular structures in the fibrous papule usually display large and irregular lumina without prominent endothelia, whereas in multinucleate cell angiohistiocytoma there is a proliferation of well-developed capillaries.

Early lesions of Kaposi's sarcoma can be differentiated from multinucleate cell angiohistiocytoma by the presence of slit-like vascular lumina, extravasation of red cells and siderosis.[7] Pseudo-Kaposi and histiocytoma may be confused clinically with multinucleate cell angiohistiocytoma, but these conditions are easily distinguished by their histology.[8] The acral arteriovenous tumour, which must be considered in the differential diagnosis, can be identified by the presence of prominent thick-walled vessels in the dermis.[9]

The vascular component of multinucleate cell angiohistiocytoma is clearly characterized by the majority of the vessels representing capillaries and metarterioles. The multinucleate cells most closely resemble fibroblasts and the different cells types, seen under the electron microscope, resemble transitional stages between activated fibroblasts and fully developed multinucleated giant cells. Similar bipolar and tripolar, occasionally multinucleated cells may be found in gingival hyperplasias[10] or in X-ray damaged skin.[11] The expression of vimentin and the absence of histiocyte–monocyte surface markers indicate fibroblasts. The weak positivity for factor VIII related antigen in fresh frozen sections is not diagnostic as factor VIII related antigen has also been observed in dendritic dermal cells in severe stasis dermatitis.[12] The presence of the zonula nucleum limitans and the prominent rough endoplasmatic reticulum are also suggestive of a fibroblastic differentiation.[13] Similar ultrastructural results have been reported in fibrous papule of the nose.[14,15] However, the occasional observation of endocytotic activity suggests histiocytic features.[16] The relationship of the cell type found in multinucleate cell angiohistiocytoma to the so-called dermal dendrocyte[17] remains to be determined.

Multinucleate cell angiohistiocytoma can be considered a distinct entity with peculiar clinicopathologic features. The lesions are benign, treatment is not necessary and surgical excision should be performed only on request.

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Sweet's syndrome and malignancy: a case associated with multiple myeloma and review of the literature

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SUMMARY

A case of Sweet's syndrome developed as a presenting feature of multiple myeloma. This is the third such case reported, suggesting that myeloma is among the increasing group of neoplasms in which Sweet's syndrome may occur as a paraneoplastic phenomenon.

Acute febrile neutrophilic dermatosis was first described by Sweet in 1964 as a benign disease of unknown aetiology.[1] Apart from minor respiratory infections the syndrome did not initially appear to be associated with other pathology. The cardinal features were fever, neutrophil polymorphonuclear leucocytosis and painful erythematous plaques affecting the limbs, face and neck which on histology showed a dense dermal infiltration with neutrophils.

Subsequently, it became clear that identical cutaneous eruptions may occur as a paraneoplastic phenomenon and it has been recommended that all cases of Sweet's syndrome should be followed up for at least 5 years, in case a malignancy should develop.[2] We report here a case of Sweet's syndrome which was the presenting feature of multiple myeloma.

CASE REPORT

A male patient was first seen in May 1988 at the age of 51 with a tender rash of 4 weeks duration over the dorsa of both hands. Otherwise, he felt entirely well. Examination revealed an erythematous eruption consisting of tender, plum coloured erythematous plaques with raised indurated margins. There were no other abnormal findings.

Histology of the involved skin revealed dense polymorphonuclear infiltration and leucocytoclasis in the dermis. A full blood count and differential white cell count were normal except for the presence of a mild normochromic anaemia; haemoglobin 10·7 g/dl. The plasma viscosity was markedly elevated at 3·74 cp (1·5–1·72). Plasma protein electrophoresis revealed a large

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TABLE 1. Malignancies associated with Sweet's syndrome

Neoplasm	Cases reported	References
Acute myelogenous leukaemia	21	2, 6–22
Other leukaemias	10	23–31
Myelodysplastic syndromes	6	15, 32–35
Multple myeloma	3	4,5
Miscellaneous myeloproliferative	6	13, 20, 36–39
Non-Hodgkin's lymphoma	2	40, 41
Carcinomas	6	13, 42–46
Total	54	

TABLE 2. Malignancies developing on follow-up of Sweet's syndrome

Reference	Malignancy	Interval*	Notes
Ilchyshyn[28]	CLL†	7 years	Paraprotein detected at presentation, which increased in level on follow-up. Recurrent episodes of Sweet's syndrome.
Lomuto[35]	Myelodysplasia	3 years	Numerous relapses of Sweet's syndrome. Anaemia on presentation which persisted.
Szabo[41]	Non-Hodgkin's lymphoma	18 months	Steroids required throughout to control Sweet's syndrome. Anaemia on presentation.
McCarthy[22]	AML†	13 months	No information regarding investigation performed on diagnosis of Sweet's syndrome.
Vestey[2]	AML†	1 year	Persistent leucocytosis throughout. Anaemia on presentation (ascribed to iron deficiency). Initial histology of the skin suggestive of leukaemic infiltration.
Tikjob[18]	AML†	5 months	Anaemia and thrombocytopenia from presentation
Apted[5]	Myeloma	3 months	Raised ESR. Sweet's syndrome persisted throughout, requiring prednisolone.
Spector[10]			
Case 1	AML†	2 weeks	Anaemia at onset
Case 2	Myelofibrosis	4 years	Recurrences of Sweet's syndrome throughout. Persistent leucocytosis.
Goodfellow[9]	AML†	4 months	Persistent anaemia and leucocytosis, Prednisolone required throughout to control Sweet's syndrome.
Matta[31]			
Case 3	Acute leukaemia	1 month	Persistent anaemia.
Case 5	Acute leukaemia	7 months	Anaemia on presentation.
Shapiro[42]	Carcinoma of tests	1 month	Recurrent episodes of Sweet's syndrome until excision of tumour. Muscle weakness.

* Interval before diagnosis of malignancy.

† CLL = chronic lymphocytic leukaemia, AML = acute myeloid leukaemia.

[illegible]

monoclonal band typed as IgG κ. The serum [illegible] showed both intact IgG κ and free κ light chains. A skeletal survey showed [illegible] the skull. Marrow aspiration and trephine biopsies from the [illegible] with abnormal plasma cells that constituted 15% of the cells present. Erythropoiesis and granulopoiesis were reduced and megakaryocytes severely decreased in number.

Treatment was commenced with prednisolone 25 mg daily and after 1 week the cutaneous lesions had shrunk considerably in size and were not tender. He then received combination chemotherapy with adriamycin, carmustine, cyclophosphamide and melphelan. His rash resolved and did not recur.

DISCUSSION

This patient demonstrated the characteristic clinical and histological features of Sweet's syndrome. Fever and a neutrophil polymorphonuclear leucocytosis were not present, but these are no longer regarded as constant features of the syndrome.[3] Results of investigations performed on presentation were diagnostic of multiple myeloma. It would therefore seem likely that Sweet's syndrome developed in this patient as a paraneoplastic phenomenon. Apart from the cutaneous eruption he was otherwise well. There were no additional findings on examination to suggest an occult malignancy and the diagnosis of myeloma might have been considerably delayed had the cutaneous manifestations not developed and been investigated.

This presentation of multiple myeloma was first reported in 1978[4] and again in 1984.[5] In both of these cases Sweet's syndrome was the presenting feature leading to a diagnosis of multiple myeloma which was of the IgA type. In view of the occurrence of this third case it would seem increasingly unlikely that this is a chance association. Furthermore, the association is no longer confined to IgA myeloma, since our patient had a paraprotein of the IgG type.

There have now been 54 malignancies (including this case), reported in association with Sweet's syndrome (Table 1). The strongest association is with acute myelogenous leukaemia. The carcinomas were all single cases originating in the testis,[42] rectum,[43] vagina,[44] ovary,[45] prostate[13] and an adenocarcinoma of unknown primary origin.[46] The patient with prostatic carcinoma also had acute myelogenous leukaemia. Curiously, all the reported primary carcinomas were pelvic in origin.

When not associated with maligancy, Sweet's syndrome affects predominantly females. Sweet[47] in 1978 reported that only one patient in a series of 15 was male, and in another series of 18 cases there was only one male.[48] It is, therefore, rather striking that 29 of the associated neoplasms (over 50%) occurred in male patients, including the case reported here. This suggests that suspicion of malignancy should be higher in a male patient with Sweet's syndrome.

The increasing number of reports indicate that Sweet's syndrome can develop as a paraneoplastic phenomenon. Two questions arise; firstly, how thorough should investigation be at presentation and secondly, how long should patients be followed up in case a malignancy should subsequently develop?

Fortunately, the task of screening for maligancy is simplified by the fact that the vast majority of associated neoplasms have been of a haemopoietic, plasma cell or lymphoid nature. Investigations can, in most cases, be limited to a full blood count, plasma viscosity and plasma protein electrophoresis.

Of the 54 malignancies reported in association with Sweet's syndrome, 21 had already been diagnosed prior to the development of the dermatosis and 20 were diagnosed at the time of [illegible] leucocytosis or raised ESR, or a prolonged course or recurrence of the dermatosis ar [illegible] which indicate a need for careful follow-up.

[illegible] investigations recommended above had returned to [illegible]

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A case of infantile digital fibromatosis showing spontaneous regression

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SUMMARY

A case of infantile digital fibromatosis (IDF) in a 3-year-old girl is reported. Two tumours were present on the left fourth toe and one over the lateral aspect of the left heel. Histological examination revealed that the tumour cells contained intracytoplasmic inclusion bodies, characteristic for this condition. The tumours showed spontaneous regression without therapy.

Infantile digital fibromatosis (IDF) is a rare, benign condition, first described in 1965.[1] This tumour is considered to be a distinct entity among the juvenile myofibromatoses.[2–5] We report a case of IDF which, within a period of 3 years, showed remarkable regression of the lesions.

CASE REPORT

A 3-year-old girl was referred to the dermatology clinic, Yokohama City University Hospital, in June 1986. She had multiple small nodules on the left foot which were firm, smooth and painless. Two were on the left fourth toe and measured 0·8 cm and 0·1 cm in diameter, respectively, and another was on the lateral aspect of the left heel, being 1 cm in diameter (Fig. 1). A biopsy of the lesion on the heel was performed and histologically, the tumour was composed of elongated, spindle-shaped fibroblasts arranged in whorls or interdigitating sheets, with abundant collagen bundles (Fig. 2). Faintly eosinophilic intracytoplasmic spherical inclusion bodies were seen on staining with haematoxylin–eosin. These also stained bright red with Masson's trichrome and purple with phosphotungstic acid-haematoxylin (PTAH), and yellow with van Gieson stain.

Immunohistochemistry for vimentin, desmin and keratin was performed using the peroxidase–antiperoxidase complex (PAP) method. Positive staining for vimentin was noticed in the cytoplasm of the tumour cells, whereas desmin and keratin were negative.

All three tumours grew slowly after biopsy (Fig. 3). However, in December 1986, regression of the tumours became apparent and by June 1988 the tumours were only small macules (Fig. 4).

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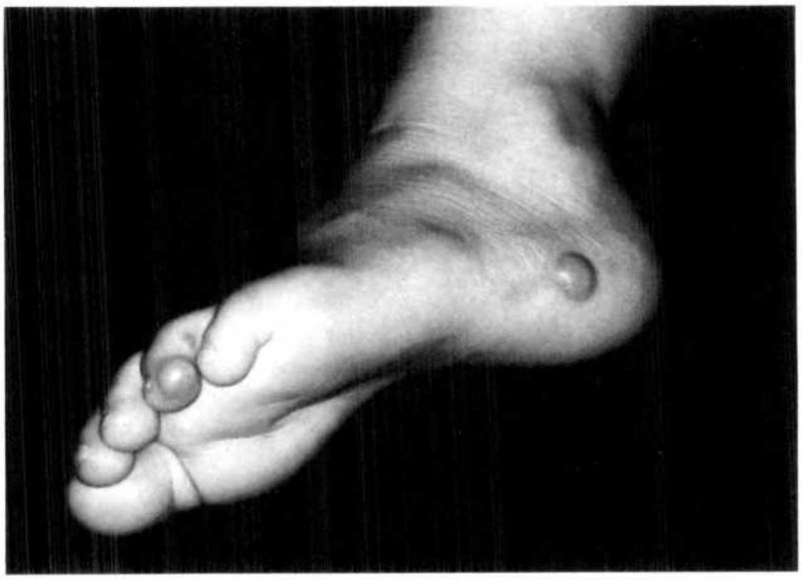

FIGURE 1. Two nodules on the left 4th toe and one on the lateral aspect of the left heel: June 20th, 1986.

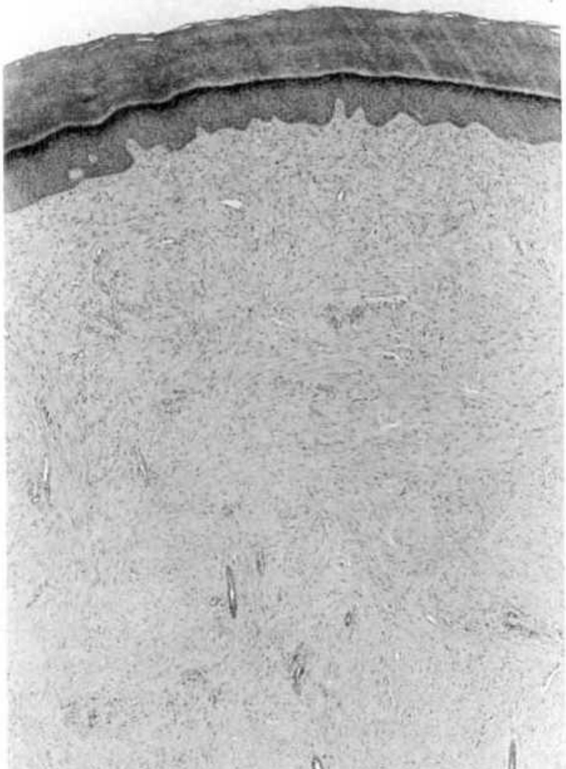

FIGURE 2. Vertical section through the tumour, showing elongated, spindle-shaped fibroblasts. (H.E. stain, × 40).

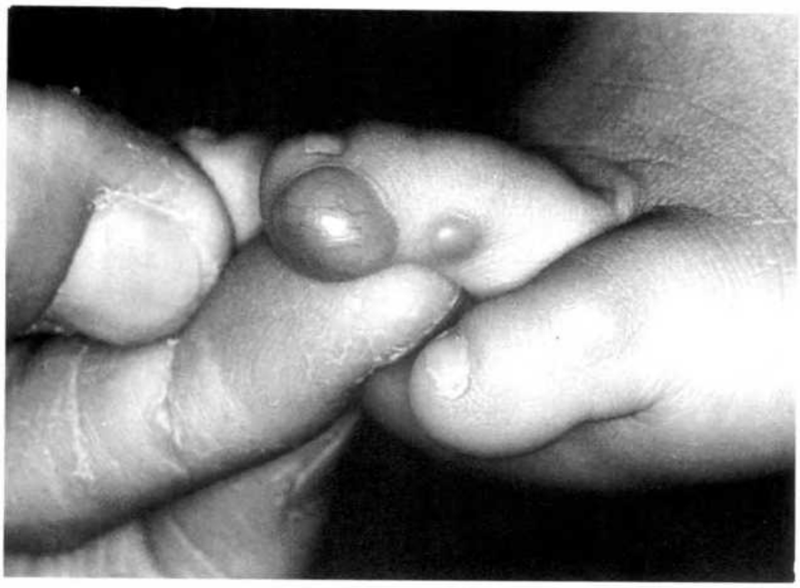

FIGURE 3. Tumours on the left 4th toe showing growth: December 2nd, 1986.

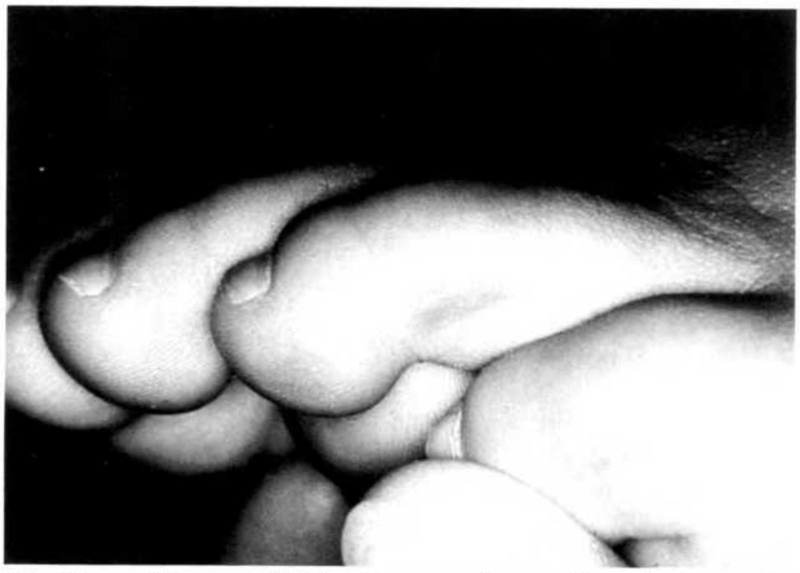

FIGURE 4. Regressed tumors on the left 4th toe, appearing as small macules: June 14th, 1988.

DISCUSSION

IDF has distinct clinicopathological features and can be separated from other forms of fibromatosis. It appears shortly after birth or within the first few years of life and both sexes are equally affected. There may be single or multiple lesions that occur on the fingers or toes. The tumours grow slowly and may adhere to the deeper tissues and there is a marked tendency for recurrence and spontaneous regression. In some, the growth may lead to deformity of the digit.

TABLE 1. Summary of IDF cases showing spontaneous regression

Papers (Reference)	Age	Sex	Site affected	Clinical course
Bean[14]	9M	F	lt. 5th toe	Spontaneous regression 2 months after partial resection.
McKenzie *et al.*[15]	9M	M	lt. 3rd, 4th, 5th toes	Recurrence 4 months after resection. Spontaneous regression in following year.
Iwasaki *et al.*[16]	0M	M	rt. 2nd, 3rd toes	Recurrence after resection. Spontaneous regression in following year.
Bloem *et al.*[17]	3M	F	rt. 3rd finger	Recurrence after resection. Spontaneous regression in 4 years.
Coskey *et al.*[18]	18M	F	lt. 3rd, 4th toes	Spontaneous regression after 5 months.
Present case	3Y	F	lt. 4th toe and left heel	Spontaneous regression in 3 years.

No instances of dissemination or distant metastasis have so far been reported. Incomplete surgical excision of these non-encapsulated lesions is followed by further growth, but spontaneous regression with time is the rule.

The histologic and electron microscopic features of IDF are unique and easily recognized by the presence of eosinophilic inclusion bodies, which have not been found in other fibromatoses. At the light microscopic level, the 1·5–10 μm round to ovoid inclusion bodies that show similar staining properties to muscle fibrils, appearing pink with H.E., red with Masson's trichrome, purple with PTAH, and yellow to orange with van Gieson are seen.

The peculiar inclusion bodies in IDF have been regarded as representing a viral infection.[1,6,7] However, the results of co-cultivation of cultured cells of this tumour with human embryonic lung cells made this unlikely[3] and ultrastructural studies failed to show any structure that could be considered to represent a virus.[5] The inclusion bodies were found to consist of packed fibrillar and granular material.[8]

Previous ultrastructural studies have revealed that the tumour cells are myofibroblasts.[2,3,5,8] The findings in an immunohistochemical study of the tumour of actin, myosin, actomyosin and vimentin[5] seems to confirm myofibroblastic origin of the cells. Although actin and vimentin have no specificity and exist in a wide variety of cells, both proteins are frequently demonstrated in myofibroblastic cells.[9-12] The localization of myosin and actomyosin is also indicative of the muscular character of myofibroblasts.

Our immunohistochemical study showed typical staining for vimentin but not for desmin. A possible reason for this is that the paraffin-embedded block was made one and a half years before the histochemical staining was performed, and the antigenicity of desmin may disappear over such an extended period. Also, muscle fibres do not always show desmin staining.[13] We have followed up our patient over the past 3 years and there has been a gradual regression of the tumours. Up to now, only five cases of IDF have been reported in the literature showing spontaneous regression (Table 1).[14-18] Although there is a marked tendency for recurrence, spontaneous regression after partial resection has been reported. We emphasize that the treatment of IDF should be conservative, particularly since these lesions do not metastasize.

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Autoimmune progesterone dermatitis responding to Tamoxifen

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SUMMARY

A case of autoimmune progesterone dermatitis is described. Exacerbation occurred premenstrually and after intramuscular and oral challenge with synthetic progesterone. The condition failed to respond to oestrogen, but there has been a marked improvement with the anti-oestrogen drug Tamoxifen.

Autoimmune progesterone dermatitis describes an eruption which is influenced by the hormonal changes of the menstrual cycle. Recurrent premenstrual flares occur which correspond to the post-ovulation rise in serum progesterone. Evidence for an allergic sensitivity to endogenous progesterone can be demonstrated.[1]

CASE REPORT

A 36-year-old woman had suffered from severe recurrent hand eczema since the age of 16 years. Menarche occurred at 15 years and the onset of her eruption appears to have coincided with the taking of hormone pills that were prescribed to regularize her periods.

The eczema always affected the wrists, hands and right knee, and occasionally other sites, being at times vesicular and at other times dry and lichenified. The eruption followed a cyclical pattern, worsening during the latter half of the menstrual cycle, with a premenstrual peak and a tendency to improve spontaneously with the onset of bleeding. Figure 1(a) and 1(b) show the relationship between severity of eczema, serum progesterone and menses. The eczema was graded on a scale from 1 to 10, and charted daily by the patient over several months before and during Tamoxifen therapy. The eruption cleared completely for the first time during her first pregnancy, only to relapse aggressively within days of delivery, becoming generalized and requiring in-patient treatment. There was some improvement during the second pregnancy but again a flare immediately post-partum. The eczema was remarkably resistant to conventional treatment, and by the time the patient was referred, she was requiring continuous oral

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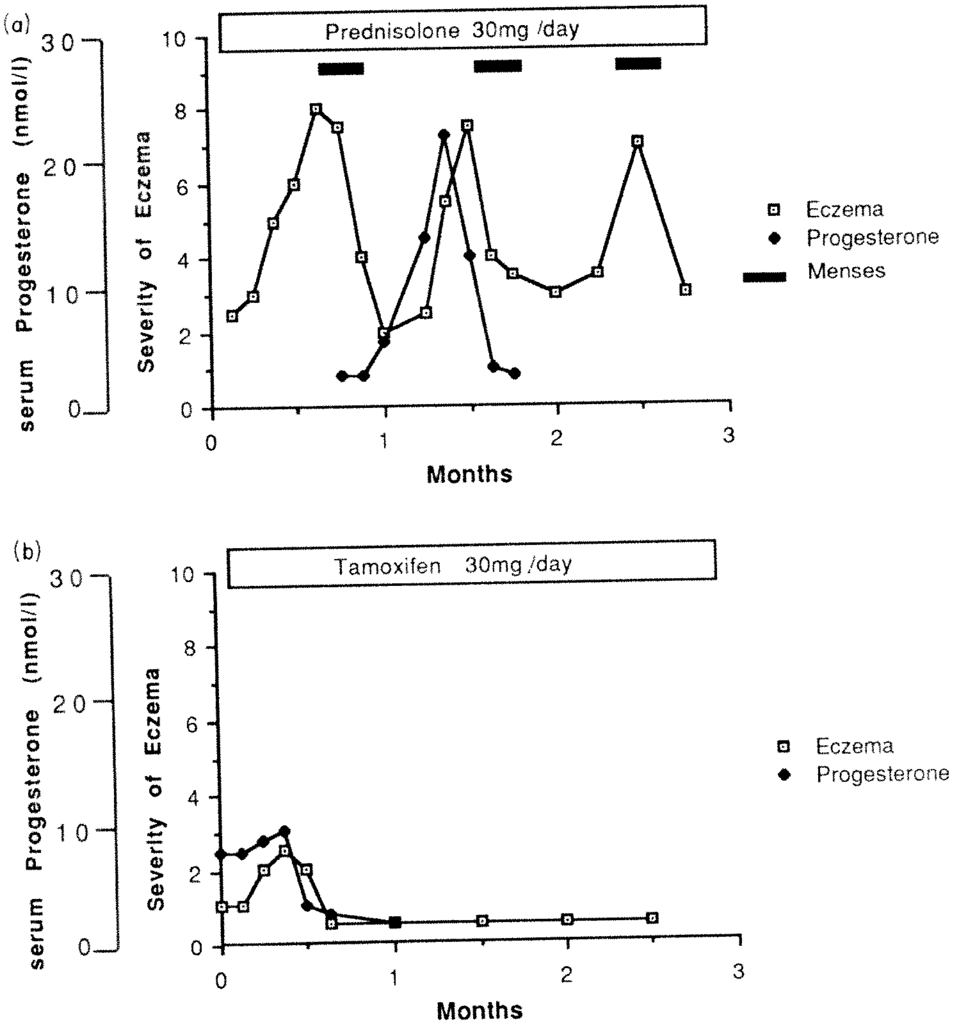

FIGURE 1. (a) Relationship of severity of eczema, (on a scale from 1–10), to serum progesterone levels and menses (b) Relationship of severity of eczema, (on a scale from 1–10), to serum progesterone levels while taking Tamoxifen.

prednisolone 30 mg/day, with potent topical steroid under occlusion to the hands with, at best, only moderate control.

A skin biopsy from the wrist showed a mild, non-specific dermatitis. Direct immunofluorescence of the skin biopsy was negative. A 3 month course of oral progesterone caused a definite deterioration in the eczema, and there was no improvement with a high dose oestrogen pill taken for 4 months. Intradermal tests with a synthetic progesterone were negative, but an intramuscular challenge produced a flare of her eczema within 4 days.

The anti-oestrogen Tamoxifen 10 mg b.d. given orally produced a definite improvement, and when increased to 30 mg/day, produced complete clearance with amenorrhoea. After 3 months a maintenance dose of 10 mg/day was given, with the patient menstruating. Her eczema was negligible and easily controlled. Progesterone levels during Tamoxifen therapy were markedly suppressed, and any slight deterioration in the eczema still coincided with the premenstrual rise in this hormone. (Fig. 1(b)).

DISCUSSION

Autoimmune progesterone dermatitis is well recognized though rare, and is characterized by the recurrence of an eruption in the luteal phase of the menstrual cycle. Twenty-four cases of autoimmune progesterone dermatoses have been reported to date in the English literature. The eruption typically has its onset in early adult life, always after menarche, and occasionally after a normal pregnancy. A history of atopy is present in less than half of the cases. As early as 1945 convincing evidence was shown for an allergic sensitivity to endogenous hormones, particularly progesterone, as the cause of a number of conditions related to menstruation and the menopause.[1] All subsequent case reports support this theory, by showing either positive cutaneous reactions to intradermal progesterone, exacerbations of the eruption by progesterone, or evidence for the presence of progesterone antibodies in suspected cases. The condition is frequently associated with prior exposure to synthetic progesterones. In a review of seven cases it was suggested that exogenous progesterone is sufficiently antigenic to act as a stimulus for antibodies, which then cross react with natural progesterone premenstrually and perpetuate the immune reaction.[2] There are two other reports of spontaneous improvement or clearance of the eruption during pregnancy.[3,4] Pregnancy is known to ameliorate many allergic states and it is suggested that there is a low maternal immunological reaction during pregnancy, probably due to the elevated cortisol levels that also occur.

The condition responds poorly to conventional treatment, irrespective of clinical type, but the majority of cases respond well to conjugated oestrogens. Prolonged clearance in a resistant case was shown after oophorectomy.[5] There is another report of a case of autoimmune progesterone dermatitis responding to Tamoxifen.[6] The mechanism of action of this drug is not entirely clear. It appears to have a peripheral anti-oestrogen effect, and by interfering with the pituitary/hypothalamic feedback mechanism suppresses ovulation, and hence the post-ovulatory rise in progesterone.

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Obituary

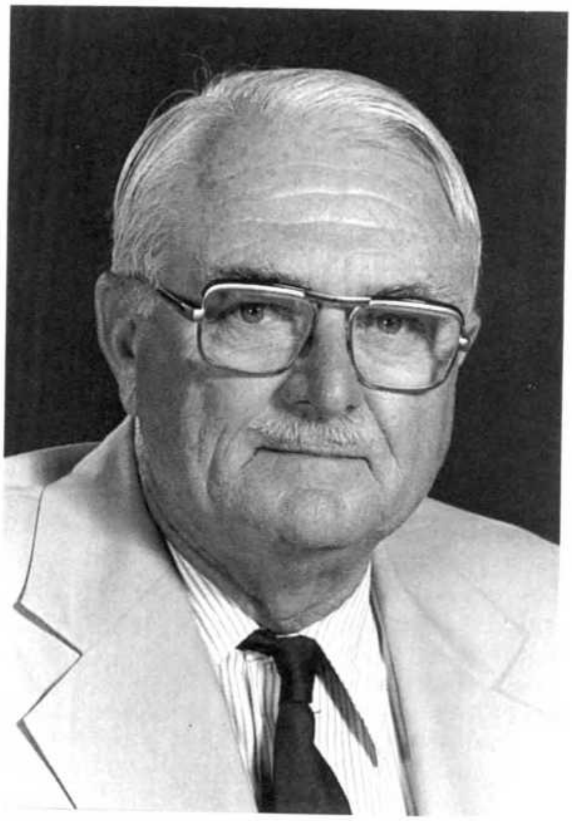
Dr Adrian M.Johnson, CBE, FRACP, FRACR, FACD

Dr Adrian Mackey Johnson died on the 3rd of November, 1988 at the age of 72. He bore his last illness with a mixture of stoicism and equanimity which was so characteristic of him.

He was born and brought up in Sydney, his father having graduated in medicine in Dublin in 1880 and commenced practice in Sydney in 1886. Of the five Johnson children, the three older boys followed their father into the practice of medicine. The youngest son became a pharmacist, and their sister, Sylvia, spent many years as a senior administrator in the Department of Pathology at the University of Sydney.

In 1933, Adrian entered the Faculty of Medicine at Sydney University. He is said to have entered undergraduate life with verve, joining in many of the activities including those of the Sydney University Regiment. For some years, he edited the University Medical Journal. He enjoyed life to the full and, as was to be expected, graduated with honours. He was appointed Resident Medical Officer at the Royal Prince Alfred Hospital, an institution which he served faithfully throughout his clinical life.

By the time war was declared in September 1939, he had almost completed his internship. He volunteered for service in the Army, and was posted as the Regimental Medical Officer to a field artillery unit in the Australian Imperial Forces. In this capacity, he served for two years in India, Egypt, Palestine, Lebanon and Syria before being invalided home with a very severe attack of amoebic hepatitis. He later described this period as the most fulfilling part of his professional life.



It was during his period of service in the Middle East, that he lead a search party which found and recovered his long time friend, and brother officer, who had been severely injured in a forward observation post. That officer, now Sir Roden Cutler VC, in his valedictory address at Adrian's funeral said "Had it not been for his dedication as the Regimental Medical Officer, and his qualities as a friend during the war, I would not be here today. It takes a special kind of man to risk his own life to save that of another. Adrian Johnson was that kind of a man."

In 1942, Adrian was evacuated to Australia. He spent the next 12 months in and out of military hospitals undergoing the not so pleasant treatment for his disorder. It was during this period that he decided to study dermatology.

It was also at this time that he proposed to Margaret Flood-Nagle, who was studying nursing at St Vincent's Hospital in Sydney. In his own words "She decided not to finish, but to devote her efforts to my welfare, with lasting good results." They were married for 46 years, and produced a family of four children: Jenni, Norman, Elizabeth (Wiz) and Rosemary (Bud).

There was no formal residency course in dermatology in Sydney at that time, and so most of Adrian's postgraduate study was pursued at a personal level with the guidance of such people as E.H.Molesworth, Grant Lindeman, Clive Robinson and other practicing dermatologists. He eagerly accepted radiotherapy training under H.J.Ham. This association nurtured the close relationship between dermatologists and radiotherapists which existed in Sydney in subsequent years.

In 1945 John Belisario was appointed part-time head and lecturer to the Sub-Department of Dermatology at the University of Sydney. He organized a Diploma course in Dermatological Medicine which Adrian undertook, and thus formalized the results of his 'do-it-yourself' training.

Adrian soon built up a large consulting practice in dermatology, and continued to serve many hospitals on a honorary basis. He re-enlisted, this time in the RAAF as a dermatologist, and continued to serve after the war as a consulting dermatologist. He retired after many years service as an Honorary Group Captain.

In 1951 the Dermatological Association of Australia was formed under the Presidency of Dr John Belisario and Adrian was asked to found and edit the Australasian Journal of Dermatology. He continued to do this in remarkable fashion for the next 25 years. He was a voracious reader with a remarkable memory. He could concentrate on a number of matters at the same time without any confusion. His long time friend, Dr Lloyd Cahill, said of him "He was the only man I have known who could devour an entire book whilst watching a day's cricket at the cricket ground and yet not miss a ball that had been bowled."

In 1952 Adrian attended the 10th International Congress of Dermatology in London. From there he set out on a grand tour of all the centres of excellence in dermatology in Europe and the United States. As a result of that journey, he made many sincere and long-lasting friendships. As a result of these friendships, and the remarkable hospitality of the Johnson family as a whole, many leading dermatologists from all over the world came to Sydney, and spent time sharing their knowledge and experience with trainee dermatologists in Australia. In reverse, many young dermatologists benefited in their travels overseas from introductions arranged by Adrian. This was one of his major contributions to the growth of expertise in dermatology in Australia.

On his return to Sydney, Adrian became involved in many other pursuits. He had always been interested in football and cricket, and rarely missed a match. He also played golf, and became interested in "The Sport of Kings".

Adrian served as a member of the Medical Registration Board of the state of New South Wales for some years, was a long time member of the Board of the Medical Defence Union of New South Wales, and a councillor of the Medical Benefits Fund (health insurance company) of Australia. He put a lot of time and effort into giving service to Legacy, a charity set up to care for the widows and orphans of service men who died as a result of their war service.

With passage of time, he was appointed head of the Department of Dermatology at Royal Prince Alfred Hospital and the University of Sydney. There he encouraged the study of dermatology, and tried to persuade many young people to combine such study with that of the basic sciences.

He had always been interested in tropical dermatology, and had travelled widely in India, Sri Lanka, New Guinea and the Pacific. His appointment as Consultant Dermatologist to the Royal Australian Air

Force enabled him to travel more widely. He had the good fortune to be in the Eastern Highlands of New Guinea, a very primitive area, at the time that Gadjusek was investigating Kuru, in the Fore people. Adrian claimed to have taken the first 8 mm movies of this condition which were later presented to the Walter and Eliza Hall Institute in Melbourne. He also paid a number of visits to Vietnam, and South-East Asia. He was appointed as Lecturer in Tropical Dermatology to the School of Tropical Medicine attached to the University of Sydney.

Adrian loved sport, history, military matters and politics. He was committed to the basic tenets of Christianity, patriotism and service to his fellow man. He was never an active politician, but was forthright in his views. He always distinguished between ideas and persons. He was determined that differences in view should never be allowed to endanger personal relationships. Hardly a day would go by without someone ringing him to ask advice or assistance. He seemed to know everyone. On one occasion, many years ago in Sydney, Professor Richard Dobson asked me in all seriousness, if there was anyone in Australia Adrian didn't know.

In 1968 he was awarded the CBE for his services to medicine and the community. This was a well deserved and much treasured honour. He was awarded an FRACP, and an FRACR. In the same year he was also elected an international honorary member of the American Dermatological Association and he was made an honorary member of the Section of Dermatology of the Royal Society of Medicine in London. Just prior to his death, he was elected as the first Emeritus Fellow of the Australasian College of Dermatologists.

We all miss Adrian very much. His last days were uncomfortable, to say the least, and yet he could always see the reverse side of the coin. Shortly before his death, he commented to Dr John Reid that there were certain advantages associated with knowing approximately "when the hour would come".

To Margaret, his widow, their children and all the grandchildren, to Sylvia his surviving sister, and to all those members of the large Johnson clan, we offer our sympathy and condolence. We also thank them for allowing us to share, in some little way, the friendship of such a remarkable man.

H.F.MOLLOY

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Polymorphonuclear leukocytes in psoriatic smokers

SIR, I was interested to read the study by Sonnex and colleagues[1] regarding polymorphonuclear leukocyte (PMN) function and its relationship to cigarette smoking in patients with psoriasis. The authors are incorrect in their statement that no information is available regarding the effect of cigarette smoking on PMN function in palmoplantar pustulosis (PPP). The results of our investigation of this relationship[2] were initially published as correspondence[3] following the publication of the epidemiological study by O'Doherty and Macintyre[4]. We examined morphology of PMN in peripheral blood films, previously demonstrated to be abnormal in psoriatic patients,[5,6] and related the results to the smoking history of patients with PPP and controls. Like Sonnex *et al.*, we found no difference *in vitro* between PMN of smokers and non-smokers; the demonstration of an *in vivo* difference in PMN chemotaxis in psoriatic smokers is therefore of considerable interest.

Sonnex *et al.* discuss the possibility that psoriatic PMN are intrinsically hyper-responsive compared to normal PMN, and that a factor in the skin or blood of smokers interacts with these PMN to increase their chemotactic responses. Cigarette smoking is recognized to cause release of a PMN chemotactic factor from lung macrophages,[7] and to increase lipoxygenase activity in platelets;[8] a similar activity in skin would provide an additional chemotactic stimulus for PMN. Furthermore, increased superoxide production by stimulated PMN[9] can in turn activate a PMN chemotactic substance in plasma.[10] Whilst these effects of cigarette smoking are potential mechanisms for increased chemotaxis of PMN in psoriatic smokers, a full explanation of their role must explain why stopping smoking does not appear to cause resolution of PPP in the reported studies.[3,4]

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Genetic control of sebum excretion and acne—a twin study

SIR, I am puzzled by the data of Drs Walton, Wyatt and Cunliffe[1] regarding acne in twins, and doubt the validity of their conclusions.

Firstly, some of the acne grades stated in Table 1 and implicit in Figure 2 are inconsistent with the grading increments of the method quoted.[2] Figure 2 shows that acne grade in eight pairs of twins differed by only 0·1 grades, yet the smallest interval between grades used by Burke and Cunliffe (1984) was 0·25. The present authors do not indicate any modification of the original method or validate this degree of accuracy.

Secondly, the population studied clearly did not suffer from acne. Although "16 out of 20 pairs in each group had evidence of acne", the median grade for all groups was 0·25 or less, on a scale of 0–10 where grades of less than 1 represent 'physiological' acne.[2] Table 1 shows that none of the 40 non-identical twins had greater than grade 1 acne (although Figure 2 mysteriously shows a female with acne 1·4 grades worse than her non-identical twin sister).

Given that the greatest difference in acne grade for any of the 40 pairs of twins was only 1·4 (which must be close to the limits of accuracy), the real conclusion from this study must surely be that there **is** concordance for acne grade in all twins. Studying a population with acne grades clustered around zero makes it difficult not to show concordance: the authors give a false impression of discordance in Figure 2 by plotting the data on a scale of 0–1·6 instead of 0 to 10, and failing to indicate realistic limits of observer error (the unexplained hatched area must have been unwittingly copied from Figure 1). Discordance can be convincingly demonstrated only by including patients with severe acne. From the report of a left–right mosaic patient with worse acne and greater sebum excretion rate (SER) on one side of the back[3] the probable answer is that acne severity, like SER, is genetically controlled and will show greater concordance in mono- than di-zygotic twins.

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Reply

SIR, We thank the Editor for allowing us to comment on the letter currently published in this issue by Dr C Moss. We concede that we should have quoted that the grading system devised by Burke and Cunliffe in 1984 has been extended. In the mid 1980s we were able to distinguish grades of 0·05, 0·1, 0·2, 0·25 and 0·375. The technique of acne grading requires much training but is a simple and reproduceable technique. We are, therefore, confident about our methods. Dr Moss quotes that the greatest difference in any acne grade for any of the 40 pairs of twins was 1·4, and comments that this must be close to the limits of accuracy. However, as indicated we can detect differences of 0·1 and we consider that our assessment techniques are not a problem.

Table 1 and Figure 2 of our paper do not represent grading increments. Table 1 is a statistical data technique table with medians and ranges based on the individual data. Figure 2 represents intra-pair differences in acne grading. It was prepared by the statisticians of Professor RM MacKie from our original data and we did not see the table until the proof stage of the paper. We have supplied the Editor and Dr Moss with a copy of this data which we originally submitted to the British Journal of Dermatology. The non-identical female acne grade range should read 0–1·5 and not 0–1·0.

The only way to study a large range of patients from one centre in a relatively rare group (such as twins) was to include physiological acne. The so-called mysterious female with an acne grade of 1·5, had a non-identical sister with an acne grade of 0·1 (difference 1·4).

Again Dr Moss has misinterpreted our tables and figures. Figure 2 is plotted on a 0–1·6 scale as this is a difference between individual pairs and not acne grading. The self explanatory hatched areas in both Figures 1 and 2 represented intra-pair differences of 0 ± 0·2 and therefore, is not 'unwittingly copied'. Dr Moss also makes the assumption about discordance being related to patients with severe acne by extrapolating observations in one patient with what appears to be mosaic and unusual truncal acne. We wish to emphasize that none of our patients had received any effective therapy for their acne. Most patients with moderate or severe acne will have had some acne therapy and this will alter or modify any base line data obtained from twins with troublesome acne. We therefore thought that the study was the best that could be done under the circumstances and provided fairly clear cut results.

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Enhancement of *Candida albicans* killing activity of separated human epidermal cells by α-melanocyte stimulating hormone

SIR, We earlier reported that freshly separated human epidermal cells are capable of killing living *Candida albicans* cells.[1] This appeared to be inducible by ultraviolet light.[1] At that time we provided indirect evidence for the involvement of an epidermal cell-derived thymocyte activating factor (ETAF), similar in function and physicochemical characteristics to interleukin-1. Interleukin-1, referred to formerly as leukocytic endogenous pyrogen,[2] has been proved to be an important mediator in the acute-phase response of the host defence.[3] Certain data suggest a role by α-melanocyte stimulating hormone (α-MSH) in the down-regulation of the interleukin-1 orchestrated acute-phase reaction,[4–6] and we investigated this further.

We used a method described earlier,[1] but with a slight modification. In the killing assay, the epidermal cells obtained from 10 healthy individuals were incubated with the yeast cells for 2 h only, in contrast with the previous 16 h. By this means we could minimize the multiplication of the *Candida* cells during the killing assay. Different concentrations of α-MSH (Sigma, St. Louis, MO, U.S.A.), dissolved in the culture medium, were added to the epidermal cells 1 h prior to the beginning of the *Candida* killing assay. None of the concentrations had any influence on the viability of either the yeast or the epidermal cells.

The results we obtained are surprising. α-MSH induces a drastic enhancement of the *Candida* killing by epidermal cells (Fig. 1). The enhancement observed with the optimum concentration of α-MSH (20 μg/ml) proved to be about twice as high as that induced by ultraviolet light.[1]

α-MSH has been reported to be present in the mammalian skin,[7] but the cells upon which this polypeptide hormone acts are not known. The freshly separated human epidermal cell suspension contains probably four cell types of different lineages: keratinocytes, Langerhans cells, melanocytes and perhaps Merkel cells. The involvement of Langerhans cells was excluded in our earlier work.[1] One possibility may be the Merkel cells, which are known to produce encephalin-like substances and vasoactive intestinal polypeptide in mammalian species.[8,9] However, α-MSH immunoreactivity could not be detected in these

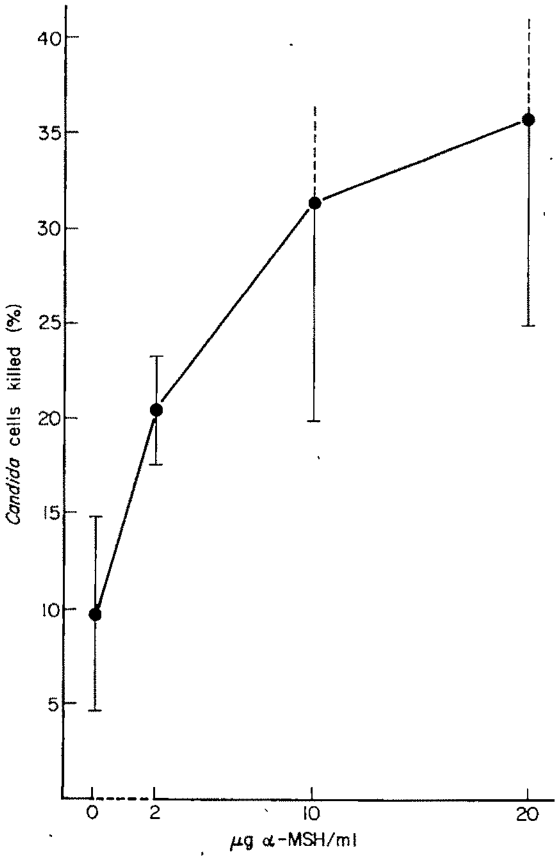

FIGURE 1. Effect of α-MSH on *Candida albicans* killing activity of human epidermal cells *in vitro*. *Candida* killing activity is expressed as the percentage of killed yeast cells. Points are means ± SD. ($n = 10$).

cells, though they were not exposed to ultraviolet radiation.[9] The role of α-MSH or biologically related substances, such as ACTH and β-lipotropin generated in the central nervous system and transported by the bloodstream into the epidermis, also requires consideration. Further investigations are needed to resolve this issue.

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Benign papular acantholytic non-dyskeratotic eruption: a new paraneoplastic syndrome?

Sɪʀ, We wish to report a unique case with multiple persistant itchy papules with pronounced acantholysis and a complete lack of dyskeratosis.

A 72-year-old woman presented in 1984 with a chronic erythroderma that had been present for about a year. The cause of the erythrodema was not established and by the beginning of 1986 she had multiple pruritic papules that were particularly located on the arms and, to a lesser extent, on the upper trunk. The papules were smooth or scaly and pink in colour and approximately 5–8 mm in diameter, some of them having a surface crust. In August 1986, the patient developed acute urinary retention due to an inoperable leiomyosarcoma of the bladder. She died several weeks later.

Biopsies from different sites of the erythrodermic skin showed the histology of a non-specific dermatitis. There was a perivascular and dermal lymphohistiocytic infiltrate and immunohistochemical studies did not reveal a T- or B-cell malignancy. Biopsies of two different papules of the papular eruption were taken for light microscopy and electron microscopy. On light microscopy suprabasal clefts due to acantholysis were present. Above the clefts there was a thick layer of keratinized and parakeratotic cells. The granular layer was thickened at the borders of the clefts (Fig. 1). The acantholytic cells were plump, round in appearance and had normal nuclei. Dyskeratosis with corps ronds and grain formation were not observed. The dermal papillae were prominent, lengthened and increased in number. A diffuse infiltrate of chronic inflammatory cells was seen in the dermis. On electron microscopy, a split was observed suprabasally in the epidermis. The basal cells remained attached to the basal lamina by normal hemi-desmosomes. There was an increase in tonofilaments which tended to aggregate around the nucleus of the basal cells. A decrease in the number of desmosomes was observed and they often showed an enlarged intercellular space. The acantholytic cells showed keratinization and degenerative changes. No corps ronds were observed. Direct immunofluorescence of acantholytic lesions was negative showing no deposition of immunoglobulin or complement.[1]

The appearance and pattern of distribution of our patient's eruption and the histological features of these acantholytic non-dyskeratotic papules is of interest. Simon *et al.*[2] presented a case with a papular eruption and with the clinical features of Grover's disease and both Darier-like histology as well as areas of spongiosis and pemphigus-like vesicles. They called this 'persistent acantholytic dermatosis'. Fawcett and Muller[3] have reported 10 patients with a chronic papular and itchy eruption and classified them as also having persistent acantholytic dermatosis. The lesions in these cases were restricted to the upper trunk and showed features of both actinic keratosis and Grover's disease and a Darier-like histology of focal acantholytic dyskeratosis.[4] They suggested that the eruption could be initiated by sunlight and discussed the existence of a possible relationship with other dermatoses with acantholysis and dyskeratosis, as seen in actinic keratosis, Darier's disease, Grover's disease and warty dyskeratoma.[5] In Grover's disease and in a syndrome that might represent a distinct entity that was classified as 'benign papular acantholytic dermatosis' (BPAD) dyskertosis can however be absent.[6] In the seven patients reported, dyskeratosis was

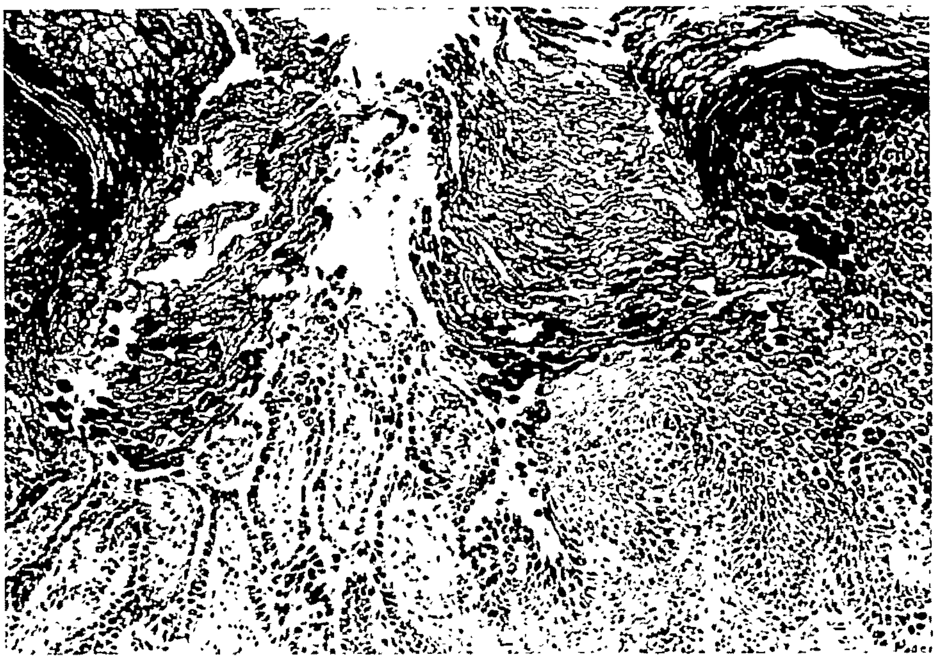

FIGURE 1. Light microscopy of a papular lesion. Acantholytic cells present without dyskeratosis (H&E × 120).

not demonstrable in five of them.[6] The papular lesions in our case resemble those of the non-dyskeratotic acantholytic type seen in Grover's disease and in BPAD.[6,7] The appearance of distinct papules (5–8 mm) with a more solitary distribution and localization in the arms in particular, would seem to differentiate the lesions in our case from those in Grover's disease and from the other cases described. In our case the term 'benign papular acantholytic non-dyskeratotic dermatosis' seems to be most appropriate. The pathogenesis is unknown, but one may speculate as to whether this unusual papular eruption represents a rare paraneoplastic syndrome.

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Razoxane and T-cell lymphoma

SIR, We report a case of T-cell lymphoma occurring in a patient with long-standing psoriasis treated with razoxane.

A 44-year-old man with psoriasis was treated with razoxane (1500 mg/week) from September 1978. In May 1984 the dose was reduced (1125 mg/week) and in December 1985 treatment with razoxane was stopped. He was commenced on hydroxyurea (1–1·5 gm/day), which he received until his presentation in May 1987 with a 6 week history of increasing wheeze and dyspneoa. Initial examination and investigations revealed a large anterior mediastinal mass, bilateral pleural effusions and splenomegaly. Full blood count showed: Hb 11·2 gm/dl, WBC 7·9 × 10^9/l (4·26 × 10^9/l neutrophils, 0·15 × 10^9/l eosinophils, 0·47 × 10^9/l monocytes, 3·0 × 10^9/l lymphocytes and 0·02 × 10^9/l poorly differentiated lymphocytes). The bone marrow aspirate was hypercellular and was infiltrated with many lymphoma cells (about 70%). Cytogenetic studies were not performed. A biopsy of the mediastinal mass showed diffuse lymphoblastic lymphoma of T-cell origin that was confirmed by surface marker studies. The cytology of the cerebrospinal fluid was normal. The bilateral pleural effusions were tapped, and the patient commenced treatment on the MRC UKALL X protocol. He is now in full clinical and haematological remission and is receiving maintenance therapy with methotrexate and 6-mercaptopurine.

An increase in the incidence of cutaneous and systemic malignancies in razoxane-treated patients is well recognized. These include squamous and basal cell carcinoma[1] and acute myeloid leukaemia.[2,3] T-cell lymphoblastic lymphoma arising in such a patient has not previously been reported. The occurrence of lymphoma in this patient may suggest that razoxane affects the pluripotential stem cell rather than solely myeloid progenitor cells. Methotrexate does not appear to increase the incidence of malignancy[4,5] and in our case has proved useful in both controlling the psoriasis and maintaining haematological remission. Regular clinical examination and cytogenetic analysis may be of value in ascertaining the onset of malignant change in razoxane-treated patients.

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Book Review

Hazards of Optical Radiation A.F.McKinlay, F.Harlen and M.J.Whillock (1988) Bristol and Philadelphia: Adam Hilger. Pp. 121. ISBN 0-85274-265-7. Price £15.00.

This is a concise guide to sources of optical radiation and their associated hazards and is written with health and safety professionals in mind. The scope of the text encompasses visible, ultraviolet, infra-red and laser radiation.

After a short introduction, there is a chapter describing the fundamental features of optical radiation. This contains definitions of measurement quantities and related terms—essential for most readers. In the following chapter, the effects of optical radiation on the skin and eye are summarized. Thereafter, the relevant safety standards are presented. These include the generally accepted limits for occupational exposure to ultraviolet radiation, visible and infra-red radiation (both photochemical and thermal retinal hazard functions), and laser radiation. Dermatologists and others who utilize optical radiation in the course of their work need to be aware of the existence of such standards, many of which relate to ocular exposure.

The bulk of the book comprises a description of a variety of optical radiation sources, including spectral emission characteristics which are considered in relation to the various exposure standards. There is here a wealth of information and it is this collation of data which ensures that this book will become a well-thumbed edition. It is a pity that the authors have included an attempt to quantify the risk of induction of skin cancer from exposure to fluorescent lighting. An upper limit of 20% and 10% is placed on non-melanoma and melanoma skin cancers, respectively. I suspect that many will share my reservations at citing such highly speculative figures. After this, there is a brief chapter on laser radiation, followed by a glossary of terms.

The text is clearly written and well supported by a mass of tabulated data. It can be recommended as a handy reference book to the expert and non-expert alike.

H.Moseley

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News and Notices

British Dermatological Surgery Group

The BDSG invite applications for travelling fellowships currently valued at £1500 each. Applicants do not need to be members of the Group and may be Consultants or dermatologists in training.

The aim of the fellowship is to encourage Dermatologists and supporting staff to visit centres of excellence either in the U.K. or abroad for further dermatological surgery experience or to attend international meetings on Dermatological Surgery. Potential applicants can obtain advice about which centres to choose from the Secretary.

Applications with a brief curriculum vitae and an outline of the proposed project should be sent to Dr Clifford Lawrence, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP. Tel: 091 232 5131. Closing date 20 June 1989.

New Concepts in Cutaneous Laser Surgery: A Hands-On Course 23–24 September 1989, Cambridge, Massachusetts

A 2-day course will be held on Saturday and Sunday, 23–24 September 1989 at the Cambridge Hyatt Hotel in Cambridge, Massachusetts. The course will be on theoretical and practical aspects of cutaneous laser surgery, and will include formal lectures, panel discussions and video demonstrations of laser techniques and laser safety. All participants will be able to acquire hands-on experience with various lasers. For further details contact The Department of Continuing Medical Education, Harvard Medical School. Tel: 617 732–1525.

Skin Pharmacology Society Sixth Annual Meeting, New York, 1–2 September 1989

The Sixth Annual Meeting of the Skin Pharmacology Society wil be held in New York, 1–2 September 1989. For further information contact: Dr Nicholas A Soter, Department of Dermatology, New York University Medical Center, 562 First Avenue, New York, NY 10016, U.S.A.

British Journal of Dermatology (1989) **121**, 153.

Erratum

Hardwick N, van Gelder LW, van der Merwe CA *et al.* Exogenous ochronosis: an epidemiological study. *British Journal of Dermatology* 1989; **120:** 229–38.

A printing error was made in this article which appeared in the February issue of the *British Journal of Dermatology*. On page 236 lines 1 and 2, under Figure 7 should read:

> 'Of the nine subjects who discontinued using hydroquinone because of hyperpigmentation, all still had evidence of ochronosis. . .'.

Lines 8 and 9 should read:

> '. . . and 4. Eight of the 53 males (i.e. 15% ± 10%) and 79 of the 142 females (i.e. 56% ± 10%) had used skin lighteners at some time. . .'

British Journal of Dermatology (1989) **121**, 155–160.

Inhibition of normal and psoriatic epidermal phospholipase A_2 by picomolar concentrations of recombinant human lipocortin I

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SUMMARY

Normal human and psoriatic epidermal phospholipase A_2 (PLA_2) was inhibited by human recombinant lipocortin I when the substrate was present in a several million-fold molar excess. Inhibition was not total, even at relatively high concentrations of lipocortin I. It is therefore suggested that human epidermis contains two species of PLA_2: one that is controlled by lipocortin I and one that is not.

The lipocortins are a family of proteins that inhibit PLA_2.[1] Lipocortin I, which is probably identical with calpactin II, calcimedin 35 and chromobindin IX[2] has been cloned.[3] It inhibits pancreatic PLA_2 only when enzyme and inhibitor are present in approximately equimolar proportions.[4–8] Hence, it has been concluded that lipocortin inhibits pancreatic PLA_2 by binding to the substrate rather than the enzyme. Similar results and conclusions have been reached using snake venom PLA_2.[7]

These results are not surprising, since lipocortins are induced by glucocorticoids, and neither pancreatic nor venom PLA_2 is thought to be under glucocorticoid control. However, human epidermis is under such control and glucocorticoid receptors have been demonstrated in this tissue.[9] We therefore studied the effect of lipocortin I on PLA_2 activity in extracts of human epidermis.

METHODS

Radiochemicals were purchased from Amersham International plc. Egg phosphatidylcholine, oleic acid, sodium taurodeoxycholate, phenylmethylsulphonyl fluoride and bovine pancreatic phospholipase A_2 were from Sigma Chemical Company, Poole, Dorset, U.K.

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Skin biopsies

Superficial skin biopsies were taken using a Davies dermatome (C.R.Thackray, Shire Oak Street, Leeds) at setting 4 from patients with psoriasis and from normal subjects, using ethyl chloride spray anaesthesia. A stainless steel guard was used to limit the size of the biopsy to 20 × 15 mm. Smaller biopsies were taken with a safety razor blade used in conjunction with a steel guard containing a 7 mm hole as already described.[10] In psoriatic patients, skin was not taken within 5 cm from the edge of the nearest lesion and was macroscopically normal. None of the subjects was on systemic therapy, nor had any used topical therapy in the 7 days prior to biopsy. Skin biopsies were taken with the approval of the North Staffordshire Health District Ethical Committee and the informed consent of the subjects.

Preparation of epidermal extracts

The skin was rinsed in 0·9% w/v sodium chloride, the epidermis separated from the dermis using 2 M potassium bromide and an epidermal extract prepared as previously described[10] with the modification that the homogenizing medium contained 1 mmol/l phenylmethylsulphonyl fluoride to inhibit endogenous proteases.

Assay of phospholipase A_2

Phospholipase A_2 activity was determined by measuring the rate of release of ^{14}C-labelled fatty acid from phospholipid. As we are interested in an enzyme that releases arachidonic acid, the most appropriate substrate would be a phospholipid containing this fatty acid in the 2 position, and epidermal phospholipase A_2 is twice as active against a phospholipid containing arachidonic acid compared with one containing oleic acid.[11] Nevertheless, we chose to use an oleic acid-containing substrate because of its greater stability. Oleic acid was included in the incubation mixture to act as a carrier for released oleic acid and ^3H oleic acid served as an internal standard. The assay conditions were chosen to achieve optimal enzyme activity as previously reported.[10]

An emulsion of ^{14}C-labelled phospholipid and ^3H-labelled oleic acid was prepared as follows: 1-palmitoyl-2-[1-^{14}C] oleoyl-sn-glycero-3-phosphocholine (5 μCi, 0·078 μg), unlabelled egg phosphatidyl choline (11·3 mg), [9,10 (n)-^3H] oleic acid (0·5 μCi, 0·025 μg) and unlabelled oleic acid (0·1 mg) were transferred to a tube in chloroform and dried under nitrogen. A solution of sodium taurodeoxycholate (150 mg) in water (5 ml) was then added and the mixture sonicated for 5 min in an ultrasonic bath. This emulsion is referred to as the substrate. For each set of assays, a cocktail was freshly prepared containing substrate (1·0 ml), 0·1 M glycine/NaOH buffer pH 8·5 (3·9 ml) and 0·5 M calcium chloride (0·1 ml). PLA_2 was assayed by incubating epidermal extract (20 μl) with cocktail (100 μl) at 37°C. After 2 h, 0·4 ml chloroform/methanol (2 : 1 v/v) was added and the free fatty acid separated by thin layer chromatography and counted as previously described.[12] Some epidermal extracts were incubated with lipocortin I for 15 min before the enzyme assay.

Protein measurement

Protein was measured as described by Lowry *et al.*[13]

RESULTS

Figure 1 shows the effect of increasing concentrations of lipocortin I on PLA_2 activity in an extract of lesion-free psoriatic epidermis. It can be seen that the PLA_2 activity does not fall to

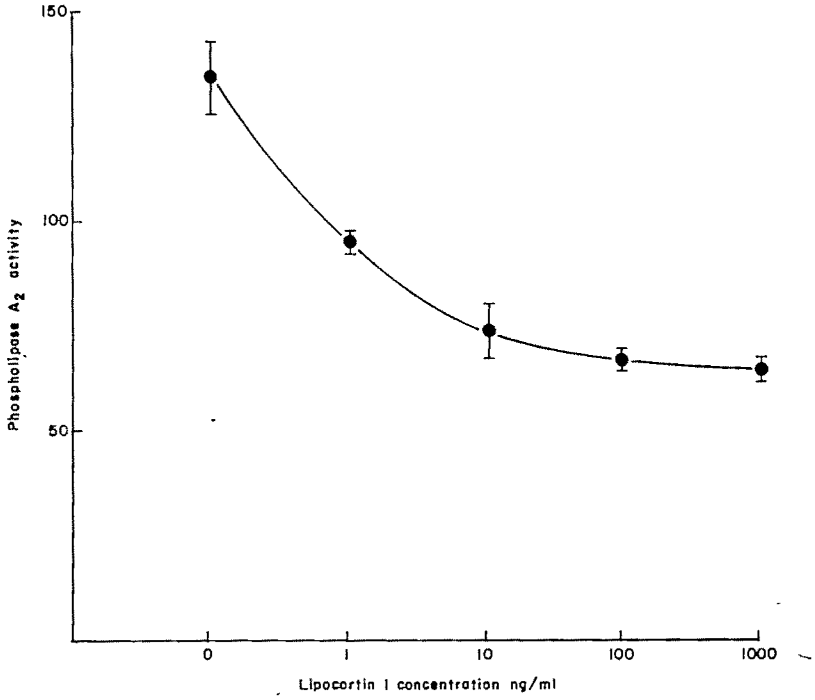

FIGURE 1. Dose response of recombinant human lipocortin I on epidermal phospholipase A_2 activity. Each incubation mixture contained in 120 μl: phosphatidylcholine (50 nmol), oleic acid (1·4 nmol), sodium taurodeoxycholate (1·15 μmol), calcium chloride (1·0 μmol), glycine/NaOH buffer pH 8·5 (7·8 μmol), psoriatic epidermal extract containing approximately 20 μg protein and lipocortin I at the indicated concentrations. Phospholipase A_2 activity is expressed as pmol fatty acid released per μg protein per h. Each point represents the mean of three determinations: bars represent standard error of the mean.

zero but levels out at a value of approximately 65 pmol fatty acid per μg protein per h. On the basis of this and similar experiments (results not shown) it was concluded that maximal inhibition occurred at a lipocortin I concentration of 100 ng/ml. Further experiments were undertaken in which epidermal extracts were assayed for PLA_2 in the presence or absence of lipocortin I at a final concentration of 100 ng/ml.

For normal epidermis, the mean PLA_2 activity was 56·5 pmol fatty acid released per μg protein per h, SEM = 3·0, $n = 16$. In the presence of 100 ng/ml lipocortin I, the mean activity was reduced to 48·7 ± 2·9, $n = 16$, a reduction which is statistically significant ($P \simeq 0{\cdot}02$, paired t-test). For lesion-free psoriatic epidermis, the mean phospholipase A_2 activity was 97·3 ± 6·7 ($n = 17$) and this was reduced in the presence of 100 ng/ml lipocortin I to 74·0 ± 8·5 ($n = 17$), a difference which is also statistically significant ($P < 0{\cdot}01$, paired t-test).

The effect of lipocortin I on the activity of bovine pancreatic phospholipase A_2 was also measured. In the first series of experiments, enzyme (final concentration 60 u/ml) was pre-incubated with lipocortin I (100 ng/ml) for various times: the enzyme activity was then determined using a 2 h incubation period. The enzyme activity in the absence of lipocortin I was 145·4 ± 4·2. In the presence of lipocortin I, but without pre-incubation, the value was 149·1 ± 1·4. Activities following pre-incubation times of 15, 30 and 45 min were 147·1 ± 2·3, 135·2 ± 0·9, and 144·9 ± 0·7. In no case does the difference between treated and control activities of the enzyme reach the conventional level of significance.

In a second series of experiments, substrate was pre-incubated with lipocortin I before adding pancreatic phospholipase A_2 and determining its activity using a 2 h incubation. In the absence of lipocortin I, the enzyme activity was 146·3 ± 3·0. In the presence of lipocortin I, but without pre-incubation, the value was 162·3 ± 6·8. Activities following pre-incubation times of 15 and 30 min were 135·9 ± 4·5 and 144·8 ± 3·1. Again, the differences did not reach the conventional level of significance.

Finally, the effect of higher concentrations of lipocortin I was investigated. The control value in this series was 82·8 ± 3·9. Following a 30 min pre-incubation with the enzyme, lipocortin I concentrations of 100 ng/ml, 10 μg/ml and 100 μg/ml yielded enzyme activities of 82·7 ± 3·7, 80·1 ± 1·1 and 82·1 ± 2·9. These differences were not statistically significant.

DISCUSSION

It has been shown that treatment of human skin with glucocorticoids both *in vivo*[14] and *in vitro*[15] reduces, but does not abolish, PLA_2 activity in epidermal extracts. The present work shows that this is also true when an epidermal extract is treated with lipocortin I.

Figure 1 shows how the PLA_2 activity in an extract of lesion-free psoriatic epidermis falls with increasing concentrations of lipocortin I. Similar results were obtained with extracts of normal epidermis, though since the initial enzyme activities were lower, the falls in activity were less. On the basis of these results, we propose that human epidermis contains two species of PLA_2; one that is lipocortin I responsive and one that is not. Recently, this view has received direct experimental support by Bergers *et al.*[16] who demonstrated two isozymes of PLA_2 in human epidermis. One isozyme is steroid responsive and is largely confined to the basal layer: the other enzyme is not steroid responsive and is located mainly in fully differentiated keratinocytes. We therefore propose that in normal epidermis, PLA_2 in the basal layer is almost completely inhibited by lipocortin, and the activity that we measure in whole epidermis comes almost entirely from the upper layers. In contrast, in lesion-free psoriatic epidermis, PLA_2 in the basal layer is only partially inhibited and the activity that we measure is the sum of the activity of the two isozymes.

Previous workers have demonstrated inhibition of pancreatic PLA_2 with approximately equimolar concentrations of lipocortin I. Davidson *et al.*[5] obtained 80% inhibition of porcine pancreatic phospholipase A_2 in a reaction mixture containing 0·16 nmol ^{3}H oleic acid-labelled bacterial phospholipid and 0·25 nmol lipocortin I. Rothhut *et al.*[7] do not specify the concentration of phospholipid in their reaction system, but Davidson *et al.*[5] estimate that it was probably less than 10 μM. Assuming this figure, one can calculate that 0·25 nmol lipocortin I in a reaction mixture containing less than 3·5 nmol phospholipid resulted in 90% inhibition of porcine pancreatic PLA_2 activity. Similarly, Fauvel *et al.*[1], using lipocortin II, obtained 95% inhibition of porcine pancreatic PLA_2 in a reaction mixture containing 0·12 nmol lipocortin II and approximately 0·5 nmol bacterial phospholipid labelled with ^{3}H oleic acid.

In contrast, our reaction mixture contained 0·26 pmol lipocortin I (assuming $M_R = 38\,500$[6]) and 50 nmol phospholipid (assuming a mean M_R for egg phosphatidyl choline of 900). Thus, maximal inhibition occurred at a molar ratio of 50 000/0·26 = 192 000 and appreciable inhibition was observed (Fig. 1) at a molar ratio 100 times greater than this. Using the assay system described, inhibition of porcine pancreatic PLA_2 was not detected even at a lipocortin I concentration of 100 μg/ml.

The fact that PLA_2 from psoriatic epidermis responded to added lipocortin I suggests that the raised PLA_2 levels, which are a feature of psoriatic epidermis[10,11,17] do not result from an inability of lipocortin to control the enzyme.

Recently, doubt has been cast on the hypothesis that lipocortin specifically inhibits PLA_2, largely because of the large concentrations of lipocortin required to inhibit pancreatic PLA_2.[18] However, Huang *et al.*[19] have suggested that different PLA_2 species may be affected differently by lipocortin I and Miele *et al.*[20] have synthesized nonapeptides, based on the structure of lipocortin I, that inhibit porcine pancreatic PLA_2 at very low inhibitor: substrate molar ratios. This paper contains the first report of a PLA_2 species which is exquisitely sensitive to a lipocortin. It is possible that PLA_2 from another steroid-responsive tissue, such as lung, will be equally sensitive to lipocortin I.

ACKNOWLEDGMENT

We thank Dr R.B.Pepinsky of Biogen Research Corporation for a generous gift of recombinant lipocortin I.

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Decreased growth inhibition by recombinant gamma interferon is associated with increased transforming growth factor-α production in keratinocytes cultured from psoriatic lesions

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SUMMARY

Keratinocytes from involved psoriatic plaques (PP), uninvolved, clinically symptomless skin of psoriatic patients (PN) and normal healthy skin (NN) have been cultured in a low calcium serum free system for multiple passages. In this way, the keratinocytes were removed from micro-environmental factors present in the skin. While the basal rate of proliferation of the PP, PN and NN keratinocytes was not different, the PP cells produced more transforming growth factor-α (TGF-α) than NN cells, and the antiproliferative response of PP cells to gamma interferon (IFN-γ), a product of activated T lymphocytes, was reduced. We studied IFN-γ because it can inhibit the proliferation of NN keratinocytes, induce their differentiation and the appearance of two immunoregulatory cell surface molecules, HLA-DR and intercellular adherence molecule-1 (ICAM-1), and because in another epithelial cell system, epidermal growth factor (EGF) modulates IFN-γ activity. The mean antiproliferative effects of IFN-γ at 50, 200, and 500 U/ml for the PP group ($n = 10$) was less compared to the NN group ($n = 11$); $P < 0{\cdot}001$, while the PN group ($n = 5$) had a less dramatic, but statistically significant, reduction in growth inhibition by IFN-γ only at 200 and 500 U/ml compared to NN cells; $P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively. The amount of TGF-α produced and secreted by PP keratinocytes from five different individuals was significantly greater than by NN keratinocyte cultures. In addition, IFN-γ induced TGF-α to a lesser extent in PP keratinocytes compared to NN keratinocyte cultures. Keratinocytes isolated from atopic dermatitis and Sézary syndrome patients were similar to NN keratinocytes.

In contrast to its differential effects on TGF-α production and proliferation, IFN-γ induced similar amounts of HLA-DR and ICAM-1 on PP, PN and NN keratinocytes. Thus, for the PP

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keratinocytes, there was a dissociation between the antiproliferative and immunomodulatory effects of IFN-γ. These results support our previous hypothesis that the hyperproliferation and altered differentiation of keratinocytes in psoriatic plaques is linked to an altered responsiveness of the keratinocytes to IFN-γ. Moreover, these results provide an *in vitro* correlate of our *in vivo* observation of increased TGF-α levels in psoriatic plaques. A new pathophysiological model to understand psoriasis is proposed which integrates these observations involving IFN-γ and TGF-α. This experimental approach also provides a system to dissect biochemical pathways of pathophysiological importance for keratinocyte hyperproliferation in psoriasis.

The hyperproliferation of keratinocytes in the epidermis has been regarded as the most characteristic feature of psoriasis.[1,2] Whether the primary cellular alteration resides in the keratinocyte, or in other cells in the skin such as fibroblasts, inflammatory cells or neurovascular cells which may produce diffusible factors that stimulate the keratinocyte in psoriasis is unknown.[3] One approach has been to isolate the epidermis and examine the inherent proliferative capacity of keratinocytes *in vitro*, apart from their cellular neighbours comprising the cutaneous micro-environment. This has produced conflicting results. Two reports using outgrowth cultures demonstrated that psoriatic keratinocytes incorporated more ^{3}H-thymidine into DNA[4] and hyperproliferated[5] compared to normal skin cells. However, subsequent to the first report, it was suggested that [^{3}H]-thymidine incorporation by cultured keratinocytes may not accurately reflect keratinocyte proliferation.[6] Another report demonstrated that keratinocytes cultured from uninvolved psoriatic epidermis had increased DNA synthesis compared to normal keratinocytes as determined by the autoradiographic labelling index.[7] At least four other reports have failed to observe any difference in the rate of proliferation when comparing keratinocytes from psoriatic lesions to normal skin cells.[8-11]

Despite the lack of a reproducible difference in cell proliferation *in vitro* between keratinocytes obtained from a psoriatic plaque (PP), or uninvolved skin from a patient with psoriasis (PN) and normal skin of a non-psoriatic (NN), Liu and Parsons suggested that there may be differences in the response of these cells to agents that inhibit cell growth.[11] One such agent that we have previously suggested may play a role in psoriasis is gamma interferon (IFN-γ), which is a secreted product of activated T lymphocytes.[12] We have reported that IFN-γ is a potent inhibitor of cultured normal keratinocyte proliferation and is an inducer of HLA-DR expression and keratinocyte differentiation.[12-15] Since IFN-γ is known to be present in psoriatic lesions[16] and since IFN-γ may be functioning as a feedback growth inhibitor in other normal cell systems,[17] it is possible that the hyperproliferation, altered differentiation and lack of HLA-DR expression in psoriatic plaques reflects an altered (decreased) responsiveness of the keratinocytes to IFN-γ in psoriasis.[18] To directly test this hypothesis, we have grown keratinocytes from 6 mm punch biopsies of PP, PN and NN skin as well as foreskin keratinocytes in a low calcium, serum free system.

Studies involving epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) were performed because EGF and TGF-α have been observed with another epithelial cell system, the thyrocyte, to be capable of inhibiting the action of IFN-γ.[19] Furthermore, TGF-α has been found to be present[20] and over-expressed at the levels of mRNA and protein in psoriatic plaques,[21] and has been suggested to be of importance in psoriasis.[22]

METHODS

IFN-γ

IFN-γ was obtained from Dr M. Shepard (Genentech, Inc., S. San Francisco, CA, U.S.A.) and had a specific activity of $1{\cdot}7 \times 10^7$ U/mg. The activity of the IFN-γ preparation utilized was continuously monitored using a sensitive and specific radioimmunoassay (Centocor, Malvern, PA, U.S.A.).

Keratinocyte culture

Punch biopsies of skin, 6 mm thick, were obtained after local anaesthesia (1% lidocaine). The biopsy sites and relevant clinical information on the psoriasis patients is listed in Table 1. Biopsies from the erythematous peripheral portion of the plaque were obtained from 10 consecutive psoriatic patients and in five of these patients, a biopsy at least 10 cm away from any lesion was also obtained and considered to represent uninvolved (PN) skin. Nine healthy adults (aged 25–58 years) without any skin disease and not using any topical or systemic medication were also biopsied. A 6 mm punch biopsy of lesional skin from a patient with atopic dermatitis and a patient with leukemic phase of cutaneous T-cell lymphoma (CTCL), Sézary syndrome, were also used as control examples of benign and malignant inflammatory dermatoses to compare to PP and PN keratinocytes. All biopsies were obtained after informed consent and with the approval of the University of Michigan Human Subjects Committee. After removal from the patient, the 6 mm punch biopsies were immediately placed in holding medium consisting of Earle's balanced salt solution (Gibco, Grand Island, NY, U.S.A.) containing penicillin/streptomycin/mycostatin (Gibco). The 6mm punch biopsy was bisected and placed in 5 ml of trypsin (0·3%), EDTA (0·1%) for 2–3 h at room temperature. After this incubation period, the epidermis was vigorously scraped with a sterile scalpel blade into 15 ml of a low calcium (0·15 mM), serum free medium (KGM, containing 10 ng/ml EGF, 5 μg/ml insulin, 0·5 μg/ml hydrocortisone, and 0·4% v/v bovine pituitary extract; Clonetics Corp., San Diego, CA, U.S.A.). It was immediately centrifuged and the pellet resuspended in 5 ml of KGM with vigorous pipetting to produce a single cell suspension which was seeded onto a single 60 mm plastic Petri dish (Lux, Flow Labs, Naperville, IL, U.S.A.). Generally, after 2–3 days small

TABLE 1. Psoriasis patient profile*

Donor	Age	Sex	Age-onset	% Body involvement	Time interval since last RX
PP1	46	M	30	25	3 months
PP2	23	M	13	50	10 months
PP3	50	M	38	80	3 months
PP4	34	F	21	60	5 months
PP5, PN5	35	M	26	60	6 months
PP6, PN6	37	M	24	70	5 months
PP7, PN7	53	F	20	60	7 years
PP8, PN8	60	F	25	60	5 months
PP9, PN9	34	F	13	75	6 months
PP10	40	M	16	85	12 months

* All biopsies of plaques were taken from the lower back and thigh regions.

clusters of adherent cells were apparent and the KGM was changed every 2–3 days with the subsequent appearance of progressively larger colonies of keratinocytes.

Two neonatal foreskins were obtained within 1–3 h of circumcision and placed in holding medium as described above. Using a Castroviejo keratome, 0·1 mm thick slices of the skin were obtained and placed in trypsin (0·3%), EDTA (0·1%) for 15–30 min at 37°C, followed by scraping of the epidermis and processing as above.

Cell proliferation studies

When the primary cultures of PP, PN and NN keratinocytes in the 60 mm dishes were subconfluent (10–14 days), they were passaged into 10 cm plastic Petri dishes (Corning, Corning, NY, U.S.A.) using 0·03% trypsin, 0·01% EDTA (5 min, 37°C). The KGM (8 ml) was replaced every 2–3 days and these cells were considered to be passage No. 1. To begin a cell proliferation assay, when the keratinocytes in the 10 cm dish were subconfluent (approximately $1{\cdot}5$–$2{\cdot}0 \times 10^6$ cells/plate), they were removed as described above and seeded (6–8×10^4 cells) into 35 mm dishes. In a typical experiment, 15 35-mm plates were seeded and, after allowing the cells to attach and spread overnight, the unattached cells ($<25\%$) and medium was aspirated and replaced with fresh KGM with or without IFN-γ. The day of adding IFN-γ was designated as day 0. Triplicate dishes containing either 0, 50, 200 and 500 U/ml of IFN-γ were maintained in a humidified incubator at 37°C for 4 days without further change of medium. It should be noted that the initial keratinocytes not seeded into the 35 mm Petri dishes were passaged and placed into 10 cm Petri dishes to maintain the culture for subsequent proliferation assays. On day 4, the medium was removed and the cells washed once with fresh KGM and the keratinocytes were detached using 0·03% trypsin, 0·01% EDTA (5 min at 37°C) and an aliquot was placed into a haemocytometer for manual counting using a phase contrast microscope as previously described.[14] The cell counts are expressed as the mean$+/-$SEM. At least six separate counts were made for each plate. At least three different experiments were performed on passage Nos. 2–8 for each type of keratinocyte (i.e. PP, PN and NN) from each donor. The NN keratinocytes (either foreskin or adult skin) were matched by passage number with the PP and PN keratinocytes and were always within 1–2 passages. The remaining keratinocytes were centrifuged and the pellet was prepared for indirect immunofluorescence staining as described below.

Indirect immunofluorescence staining/FACS analysis

The cell pellets were resuspended in PBS containing 5% FCS (Hyclone) and indirect immunofluorescence staining was performed as previously described.[13] Briefly, $0{\cdot}5 \times 10^6$ keratinocytes were indirectly stained for 20 min on ice with 0·5 μg of anti-HLA-DR monoclonal antibody TS1·16 (Gift from Drs C. Clayberger and A. Krensky, Stanford University), anti-intercellular adherence molecule-1, (ICAM-1) (RR 1/1; Gift from Dr T. Springer, Dana Farber Cancer Institute) and OKT8 (Ortho Diagnostics, Rareville, NJ, U.S.A.) diluted in heat-inactivated FCS containing 0·1% sodium azide. All of these primary antibodies are of the IgG_1 isotype. Detection of ICAM-1 was performed because we have previously observed that IFN-γ induced ICAM-1 on NN keratinocytes.[23] The keratinocytes were then washed twice and stained for 20 min with 0·5 μg of fluorescein isothiocyanate-conjugated (FITC) goat anti-mouse IgG monoclonal antibody (Tago, Burlingame, CA, U.S.A.). The keratinocytes were then washed twice more and fixed with freshly prepared 1% paraformaldehyde in PBS. The fluorescence per cells was determined using a fluorescence activated cell sorter (EPICS 54;

Coulter Corp.) and a histogram showing the number of stained cells versus the intensity of fluorescence was recorded.

Measurement of TGF-α protein

To determine the amount of TGF-α produced by cultured keratinocytes, semiconfluent keratinocytes (approximately $5–6 \times 10^5$ cells/35 mm dish) containing 1·5 ml of KGM were maintained for 48 h at 37°C, and the conditioned medium was assayed for TGF-α using a radioimmunoassay kit (Biotope, Seattle, WA, U.S.A.). Briefly, duplicate 100 μl aliquots were removed and immediately reduced and denatured as per the manufacture's recommendation. The samples were run with at least five different known TGF-α standards. No cross reactivity of the assay system for EGF was noted up to 1 μg/ml.

To determine the amount of TGF-α that may have been bound to the keratinocyte cell surface, thoroughly washed keratinocytes were exposed to a 'cold acid wash' in 50 mM glycine, 100 mM NaCl; pH 3·0 for 4 min at 4°C as previously described.[24]

Measurement of TGF-α mRNA

Keratinocytes ($2–4 \times 10^6$ per 100 mm dish) were lysed with 4 M guanidinium isothiocyanate, 5 mM sodium citrate, pH 7·0, 100 mM β-mercaptoethanol, 0·5% sodium sarcosinate, and RNA was isolated by overnight centrifugation at 100 000 × *g* over 5·7 M CsCl in 100 mM EDTA, pH 7·0.[25] RNA concentration was determined by absorbance at 260 nm and confirmed by non-denaturing agarose gel electrophoresis as previously described.[26] RNAs were size-fractionated by electrophoresis on a 1% formaldehyde-agarose gel,[25] and transferred to derivatized nylon membrane (Zeta-probe, Bio-Rad) in 10 × SSC, (1 × SSC = 0·15 M sodium chloride, 15 mM sodium citrate) as recommended by the manufacturer. Filters were hybridized against ^{32}P-labelled probes prepared by random priming[27] exactly as previously described.[28] The TGF-α cDNA probe psp65C17N3 was generously provided by Dr R. Derynck of Genentech, Inc.[22] The chicken glyceraldehyde-3-phosphate dehydrogenase (GAD) cDNA probe was derived from pGAD 28 and served to control for RNA loading and intactness.[29] Specific hybridization was estimated by subjecting the autoradiographs of the blots to laser scanning densitometry as previously described.[28]

Statistical analysis

The two-tailed Student's *t*-test for unpaired samples was performed.

RESULTS

Antiproliferative effect of IFN-γ on NN, PN and PP keratinocytes

On day 0, there were approximately $5–6 \times 10^4$ keratinocytes per 35 mm dish which increased to approximately $4–7 \times 10^5$ cells/dish by day 4 (approximately three population doublings). While there was some variation (< 20%) in the exact degree of proliferation between days 0 and 4 for all the different types of keratinocytes (i.e. PP, PN, NN-adult and foreskin), approximately three population doublings were consistently observed throughout multiple passages of the different keratinocytes, although there was a tendency for proliferation to decline in later passages (data not shown). Since there was some variation in the degree of proliferation of the different types of keratinocytes in the absence of IFN-γ, we have expressed the data for the cultures treated with IFN-γ as a relative percentage of inhibition compared to the non-IFN-γ treated cells for that particular experiment. Thus, if a keratinocyte culture had 5×10^5 cells on

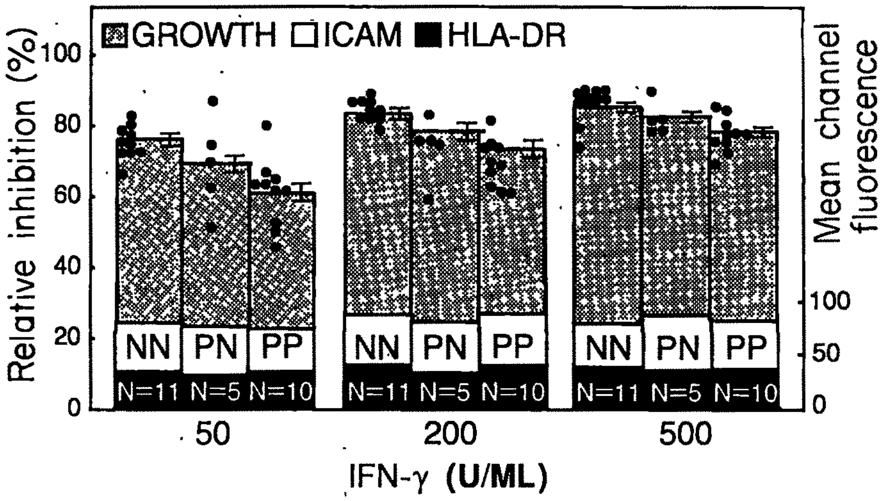

FIGURE 1. The relative growth inhibitory effects and induction of ICAM-1 and HLA-DR by IFN-γ (50, 200 and 500 U/ml) are summarized for multiple passages of all subjects on day 4 as compared to keratinocytes grown in the absence of IFN-γ. The left side vertical axis and stippled bars represent the relative percentage inhibition of growth by IFN-γ on NN, PN, and PP keratinocytes. The right side vertical axis and the open bars (ICAM-1), and bars composed of small boxes (HLA-DR) represent the mean channel fluorescence values of the relative immunomodulatory effects of IFN-γ on NN, PN and PP keratinocytes.

day 4 in the absence of IFN-γ, and had 1×10^5 cells in the presence of IFN-γ, then this would be reported as 80% relative inhibition. The degree of variation in proliferation that we observed is similar to that reported by O'Keefe and Chiu.[30] In contrast to a previous report in which foreskin keratinocytes grew more rapidly in serum containing medium than keratinocytes obtained from normal adult skin,[31] no significant difference in the degree of proliferation between days 0 and 4 was observed in our tissue culture system and therefore the two neonatal foreskin NN keratinocytes were combined with the nine adult NN keratinocyte data.

The relative antiproliferative effects of IFN-γ (50, 200 and 500 U/ml) on passage Nos. 4, 5 or 6 keratinocytes compared to the same keratinocytes grown in the absence of IFN-γ on day 4 is summarized graphically in Figure 1 for all the subjects in this study. The mean relative percentage inhibition on the NN keratinocytes by IFN-γ (50, 200 and 500 U/ml) was 76 ± 2, 84 ± 2 and 88 ± 2 respectively, which included nine adult and two neonatal foreskins and a total of 33 experiments (triplicate experiments on 11 different NN keratinocyte cultures). The mean percentage inhibition on the PP keratinocytes by IFN-γ (50, 200 and 500 U/ml) was 59 ± 2, 69 ± 3 and 79 ± 3 respectively, which included 10 different psoriatic patients. The difference between the NN and PP group mean percentage inhibition for all three IFN-γ concentrations was highly statistically significant ($P<0{\cdot}001$). The mean percentage inhibition on the PN keratinocytes by IFN-γ (50, 200 and 500 U/ml) was 68 ± 2, 77 ± 2 and 83 ± 2 respectively which included five different psoriatic patients. The group mean difference between NN and PN keratinocytes was less dramatic than with PP keratinocytes. There was no statistically significant difference between NN and PN keratinocytes at 50 U/ml ($P>0{\cdot}1$), but the differences were statistically significant at 200 U/ml ($P<0{\cdot}05$) and 500 U/ml ($P<0{\cdot}01$).

We next used the NN group mean percentage inhibition by IFN-γ (50, 200 and 500 U/ml) of 76, 84 and 88% respectively, to compare individual PP and PN cultured keratinocytes antiproliferative responses to IFN-γ. We found that in nine of 10 PP keratinocyte cultures (patients Nos. 1–6 and 8–10) and two of five PN keratinocyte cultures (patients 5 and 10), there was less growth inhibition by IFN-γ ($P < 0.001$ for PP keratinocytes; and $P < 0.01$ for PN keratinocytes vs. NN keratinocytes). The keratinocytes from the atopic and CTCL patients showed a similar antiproliferative response to IFN-γ as the 11 different adult NN keratinocytes (data not shown). This relatively greater proliferation in the presence of IFN-γ for the PP and PN keratinocytes was consistently observed when using keratinocyte cultures between passage No. 2 and passage No. 8.

Morphological appearance of NN and PP keratinocytes

With phase contrast microscopy, there were also morphological differences between NN and PP keratinocytes both in the absence and in the presence of 50 U/ml of IFN-γ. On day 0, the PP keratinocytes appeared slightly larger and less basaloid compared to NN keratinocytes (Fig. 2). On day 4, the 50 U/ml of IFN-γ treated NN keratinocytes revealed numerous foci of stratified clusters of keratinocytes with the adjacent attached cells having a differentiated appearance as previously described.[15] In contrast, the PP keratinocytes on day 4 of IFN-γ (50 U/ml) revealed a greater density of attached cells with only a rare, small cluster of cells, with the adjacent keratinocytes retaining a more basaloid, less differentiated, appearance than the comparable NN keratinocytes (Fig. 2).

Immunomodulatory effects of IFN-γ on NN, PN and PP keratinocytes

The ability of IFN-γ to induce HLA-DR and ICAM-1 expression at all concentrations was the same for PP, PN and NN keratinocytes without any statistically significant difference amongst any normal and psoriatic patients (Fig. 1). Even at 50 U/ml, the PP and PN keratinocytes expressed similar amounts of HLA-DR and ICAM-1 as NN keratinocytes. The increased expression of ICAM-1 over HLA-DR is consistent with our previous observation.[23] Thus, our data indicate a dissociation of the antiproliferative effect of IFN-γ from the immunomodulatory effect (induction of HLA-DR and ICAM-1) for the psoriasis patients.

Basal and IFN-γ inducible TGF-α production by NN and PP keratinocytes

We examined the amount of TGF-α produced by six different NN keratinocyte cultures (NN 4–9) compared to five different PP keratinocyte cultures (PP 6–10) between passages Nos. 2–5, both in the presence and absence of IFN-γ (50 U/ml). Figure 3 reveals that the NN keratinocytes (left panel) produced between 0·4–1·1 ng/ml per 10^5 cells (mean $= 0{\cdot}78 \pm 0{\cdot}29$) in the absence of IFN-γ, whereas the PP keratinocytes (right panel) produced between 1·7–5·2 ng/ml per 10^5 cells (mean $= 2{\cdot}78 \pm 1{\cdot}52$). The difference in these mean values comparing NN to PP keratinocytes is significant ($P < 0{\cdot}01$). It should be noted that all of the cultures were seeded at the same density and that the TGF-α measurements were made from 48 h conditioned media (1·5 ml of KGM).

When the NN keratinocytes were exposed to IFN-γ (50 U/ml), they produced an increased amount of TGF-α (approximately 2–3 fold) whereas the PP keratinocytes showed either no response (PP6, 7, 10) or only a slight increase (PP 8, 9). Thus, the PP keratinocytes were much less responsive to the effects of IFN-γ with respect to their increased TGF-α production compared to NN keratinocytes. This increased production of TGF-α was not due to release of cell surface bound TGF-α, since brief treatment of thoroughly washed NN and PP keratinocytes with 'cold acid', which removes cell surface molecules,[24] revealed similar amounts of TGF-α for both IFN-γ treated and untreated NN and PP keratinocytes which was approximately 10% of the amount detected in the conditioned media (data not shown).

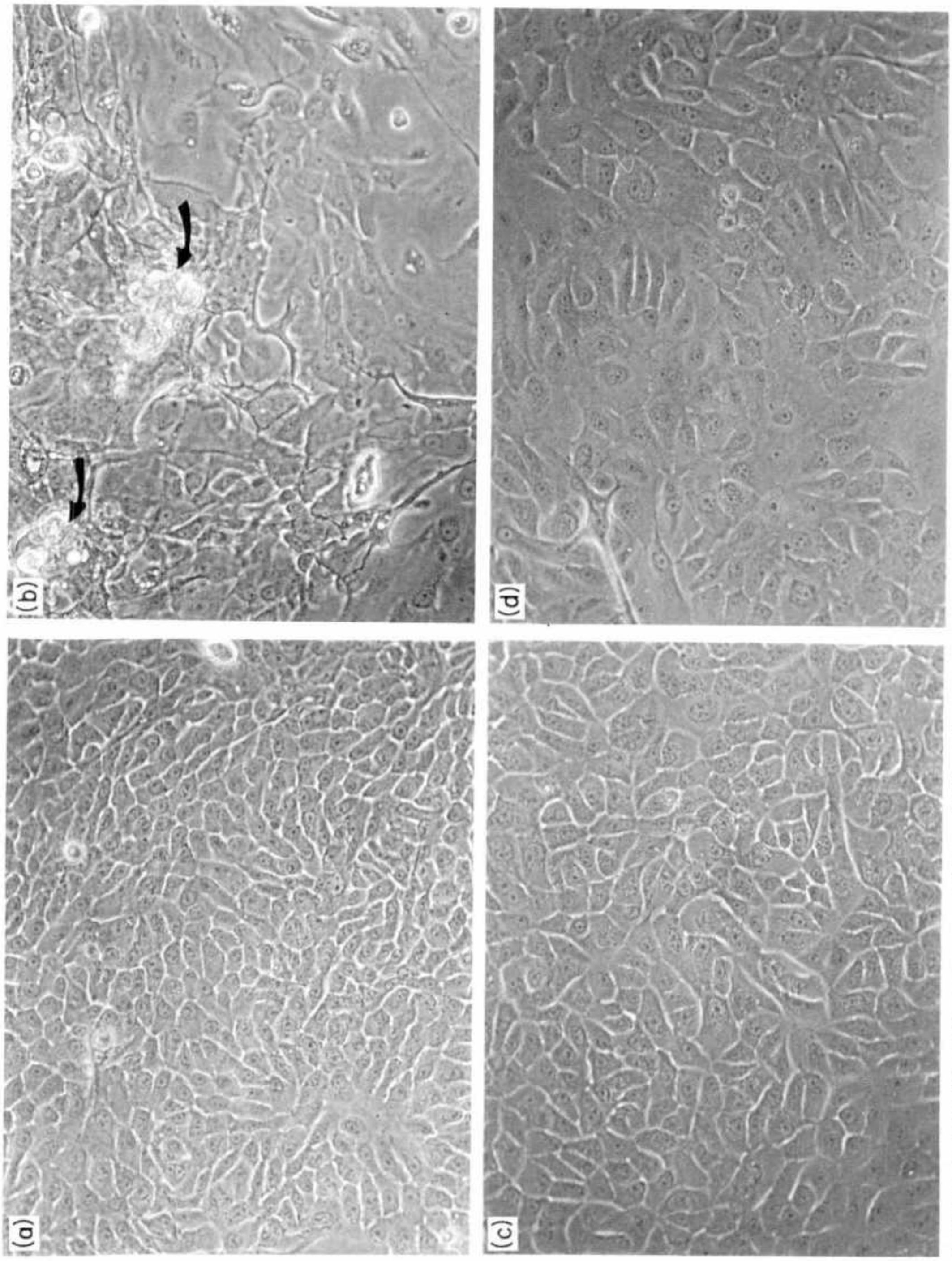

FIGURE 2. Phase contrast microscopic appearance of NN and PP keratinocytes. (a) Untreated NN keratinocytes (passage No. 4). (b) NN keratinocytes 48 h after IFN-γ (50 U/ml). Note the numerous stratified clusters of keratinocytes (arrows). (c) Untreated PP keratinocytes (passage No. 4). Note that compared to NN keratinocytes, the PP keratinocytes are slightly larger, (d) PP keratinocytes 48 h after IFN-γ (50 U/ml). Note the lack of stratified clusters compared to NN keratinocytes (× 200 mag).

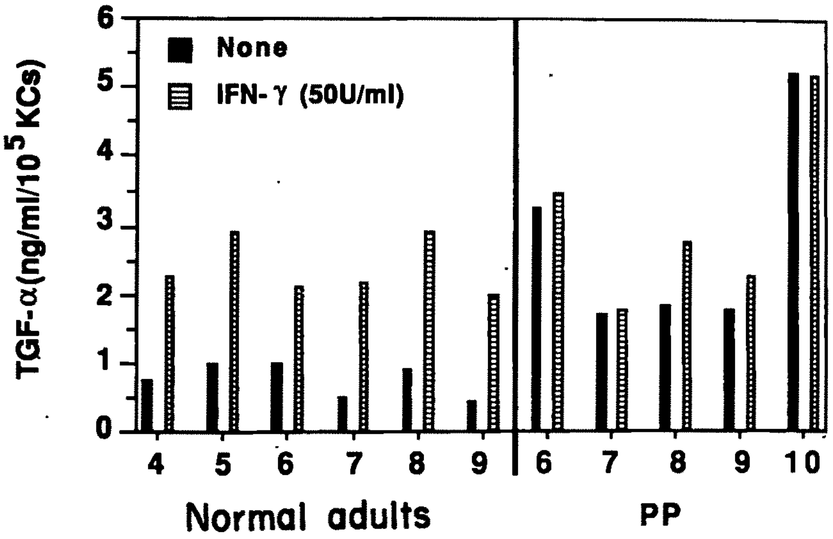

FIGURE 3. The production of TGF-α by six different NN keratinocyte cultures and five different PP keratinocyte cultured with and without IFN-γ treatment resulted in 2–3 fold increases in TGF-α production, whereas there was minimal change in the PP keratinocytes with respect to IFN-γ exposure (SEM <15%).

The increase in secreted TGF-α protein as detected by the radioimmunoassay of the 48 h conditioned medium, was paralleled by a 3·7 fold stimulation of TGF-α mRNA measured 68 h after IFN-γ treatment of NN keratinocyte cultures (Fig. 4a). Expression of the GAD reference gene varied with time over these extended intervals (Fig. 4b) but displayed little variation in response to IFN-γ (Figs. 4a–b). These results indicate that the increased level of TGF-α secreted in response to IFN-γ reflects increased gene activity as previously reported for the TGF-α gene response to EGF.[22]

DISCUSSION

Using multi-passaged keratinocytes grown on plastic, in a low calcium serum free medium, the PP keratinocytes in nine of 10 patients grew significantly better in the presence of IFN-γ, compared to either NN keratinocytes, or keratinocytes obtained from atopic and Sézary syndrome patients. Our tissue culture system is different from previous investigations which relied on primary cultures of epidermal cells, or keratinocytes grown in serum containing medium in the presence of feeder layers or collagen.[4–9] Utilization of passage Nos. 2–8 keratinocytes reduces possible effects of various diffusible growth modulating factors, which are derived from other cellular constituents in the psoriatic plaque, and retained by primary cultures of keratinocytes. The greater relative proliferation in the presence of IFN-γ which occurred in nine of 10 PP cultures and in only two of five PN cultures may reflect heterogeneity, due to either the type of keratinocyte isolated from the psoriatic plaque,[32] or isolation of different basal cell subpopulations having different *in vitro* responsiveness.[33] Our results in which differences were observed throughout multiple generations of keratinocytes raises the possibility of a genetically transmissable alteration in PP keratinocytes. This *in vitro* observation

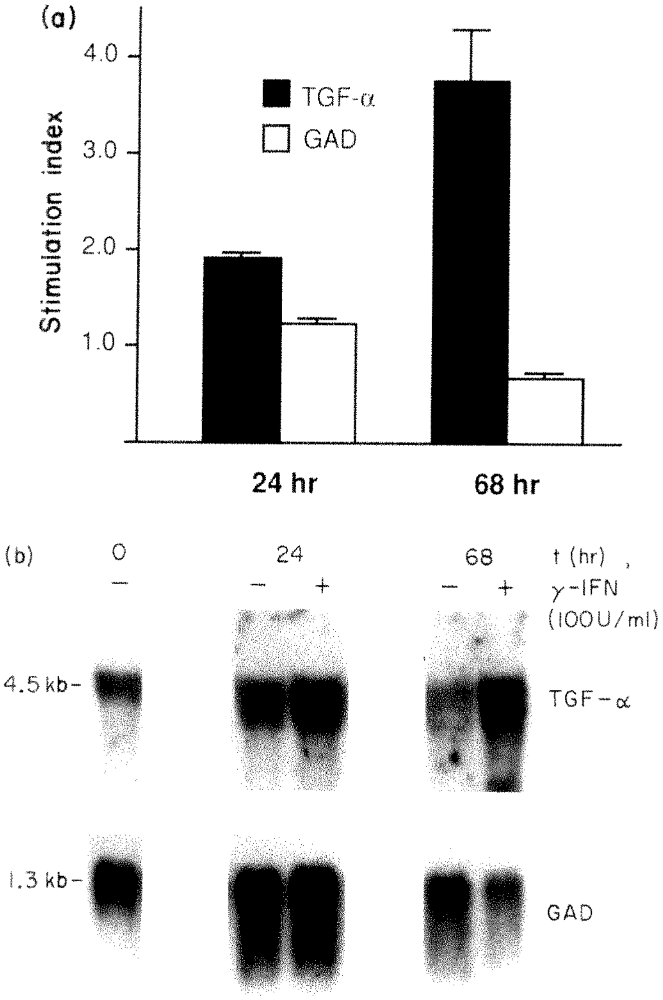

FIGURE 4. RNA blot hybridization analysis of effects of IFN-γ treatment of TGF-α mRNA levels in NN keratinocytes. (a) Densitometric analysis. Integrated autoradiographic intensities are presented as a stimulation index, defined as the ratio of the intensity in the presence of IFN-γ (100 U/ml) to that in its absence at 24 and 68 h after treatment. Results shown are the mean and range of duplicate determinations from a representative experiment. (b) Autoradiography: Only the relevant bands are shown. Upper panel, TGF-α probe. Lower panel, GAD probe. Molecular weights of the hybridizing bands are shown to the left.

is consistent with clinical and epidemiological data suggesting that genetic factors play an aetiological role in psoriasis.[34,35]

Since differences in response to IFN-γ were much more pronounced in PP than PN skin (which may have been involved in psoriasis at some time in the past), it suggests that this difference in behaviour of the keratinocytes may have been produced during the psoriatic process. It should be noted that Baker *et al.* have recently observed that keratinocytes obtained from psoriatic lesions are also less growth inhibited by IFN-γ than normal keratinocytes.[36]

They also report however, that HLA-DR expression by the psoriatic keratinocytes was less after IFN-γ exposure.[36] We currently have no explanation for this difference. Our inability to detect any difference in the ability of IFN-γ to induce HLA-DR or ICAM-1 despite the decreased antiproliferative effect on PP keratinocytes, suggests a dissociation between the immunomodulatory and antiproliferative effects in these cells.

One possible explanation for the apparent refractoriness of PP epidermis to the effects of IFN-γ involves TGF-α and its cellular receptor, the epidermal growth factor (EGF) receptor. IFN-γ-induced HLA-DR expression in cultured human thyroid epithelial cells can be suppressed by TGF-α and its evolutionary and functional homologue,[22] EGF.[19] We have recently observed that the TGF-α gene is overexpressed at the levels of mRNA and protein in psoriatic epidermis, whereas expression of the EGF gene was undetectable.[21] Increased EGF receptor expression by keratinocytes in psoriatic epidermis has been previously demonstrated by Nanney *et al.*[37] These results suggest that TGF-α-mediated signal transduction through the EGF receptor is a prominent feature of psoriatic epidermis. By analogy to thyroid epithelial cells, EGF receptor activation by TGF-α may block the ability of IFN-γ known to be present in psoriatic lesions to induce HLA-DR expression and inhibit proliferation. Our conditions of cell culture provide both normal and psoriatic keratinocytes access to saturating amounts of EGF, while keratinocytes in psoriatic lesions *in vivo* are exposed to higher levels of TGF-α *in situ* than are the keratinocytes of normal epidermis. This difference could explain the lack of differential HLA-DR responsiveness to IFN-γ in PP relative to NN keratinocytes. It is interesting that a very similar dissociation of IFN-γ effects on proliferation and HLA-DR expression has also been reported in human breast cancer cell lines, which, like keratinocytes, display EGF receptors and proliferate in response to EGF.[38] However, the presence of comparable amounts of exogenous EGF cannot explain why PP keratinocytes produced greater amounts of TGF-α than NN keratinocytes in the absence of IFN-γ.

An unexpected linkage between IFN-γ and TGF-α synthesis was discovered during this work. When NN keratinocytes were treated with IFN-γ, there was a significant increase in the amount of TGF-α produced. This increase appeared to reflect increased TGF-α gene activity, as a roughly proportional increase in TGF-α mRNA was observed in NN keratinocytes over the same time interval. In contrast, PP keratinocytes did not further increase their elevated basal TGF-α production in response to IFN-γ. Since IFN-γ is not present in our culture medium and is not produced by keratinocytes, these differences cannot be due to the presence of endogenous IFN-γ under basal conditions. We suspect that psoriatic keratinocytes possess an altered mechanism of signal transduction *in vivo* which persists under *in vitro* culture conditions, increasing basal levels of TGF-α production and inhibiting keratinocyte IFN-γ responsiveness. While the biochemical nature of this defect remains unclear, it could involve increased EGF receptor responsiveness resulting in enhanced TGF-α autoinduction.[21,22]

It is possible that spatial variation in the distribution of IFN-γ responsive vs. IFN-γ unresponsive cells may explain several features of psoriatic lesions. The lesions in psoriasis have been known to be heterogeneous both clinically[32] and histologically.[39] While some of this heterogeneity reflects the presence of epidermal appendages such as hair follicles and eccrine units, a significant contribution is also provided by local variation in the degree of keratinocyte differentiation. We speculate that keratinocytes within a lesion which contain a prominent granular cell layer and absent mitotic figures, including those of follicular epithelium,[40] are IFN-γ responsive and undergoing cellular differentiation.[15] One response of differentiated keratinocytes *in vivo* appears to be a reduction in the number of EGF receptors.[38] In addition to increased production of TGF-α, we have recently shown that IFN-γ causes a down-modulation of high affinity receptors of EGF in cultured NN keratinocytes.[41] The down modulation of the EGF receptors by IFN-γ may effectively remove a cellular mechanism responsible for negative feedback control of TGF-α production. Thus, IFN-γ responsive keratinocytes may serve as a paracrine source of IFN-γ-inducible TGF-α and yet not proliferate in response to it due to a lack of EGF receptors. In contrast, adjacent or neighbouring keratinocytes which are IFN-γ unresponsive, continue to express EGF receptors and therefore, could be driven to hyperproliferate by this induced TGF-α production from IFN-γ responsive keratinocytes.

This proposition represents a novel pathophysiological model which integrates the morphologic heterogeneity known to be present within psoriatic lesions both clinically[32] and histologically,[39] with the growth factor/growth factor receptor[20-22,37] and immunological alterations[12,42] also observed in psoriasis.

If one role of IFN-γ production by activated T lymphocytes is to function as a feedback growth inhibitor [17] regulating keratinocyte proliferation, then decreased responsiveness of the PP keratinocyte to IFN-γ may contribute to the epidermal hyperplasia so characteristic of psoriasis.[1,2] Since PP keratinocytes tended to be more growth inhibited by very high concentrations of IFN-γ (500 U/ml),[43] treating psoriasis patients with exogeneously administered IFN-γ may be therapeutically beneficial.[44] However IFN-γ promotes T lymphocyte and monocyte adherence to keratinocytes,[45] and may increase the trafficking of inflammatory cells into the psoriatic lesion,[46] and this effect may counteract its direct antiproliferative effect[14] and thus not produce any 'net' clinical response.[47] The observed lack of inhibition of PP keratinocyte proliferation *in vivo* following intralesional IFN-γ administration, despite induction of keratinocyte HLA-DR expression[47] is consistent with our current results. In patients with lepromatous leprosy, intradermal injection of IFN-γ induced a dermal inflammatory reaction accompanied by epidermal hyperplasia and keratinocyte HLA-DR expression.[48] This result also suggests that, *in vivo*, there may be a dissociation of the antiproliferative effects of IFN-γ from its immunomodulatory role. Further work aimed at understanding the cellular basis for the dissociation of the antiproliferative action from the immunomodulatory action of IFN-γ in psoriasis, as well as a greater appreciation for the diversity of functional responsiveness to IFN-γ expression of EGF receptors and TGF-α production by keratinocytes within psoriatic lesions, may also provide new insights into the pathophysiology of psoriasis.

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Can HLA-DR expression help in the routine diagnosis of malignant melanomas?

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SUMMARY

Two monoclonal antibodies, TAL-1B5 and LN3, directed at epitopes of HLA-DR were applied to a variety of benign naevi and malignant melanomas. Of the 31 naevi examined, 28 were completely unreactive. Weak staining was confined to a few cells in two intradermal naevi and there was more widespread reactivity in a halo naevus. Seven of the 25 primary cutaneous malignant melanomas were reactive with one or both of the antibodies and this reactivity was more marked in the deeper lesions. These positive cases were not felt to present diagnostic difficulties and it was concluded that TAL-1B5 and LN3 were not of use in distinguishing between benign and malignant melanocytic proliferations.

Expression of HLA-DR by malignant melanoma cells in culture was first documented by Winchester *et al.*[1] Since then a number of studies have confirmed this expression in cryostat sections,[2–5] but naevocellular naevi have been found to be unreactive.

Recently antibodies have become available that detect epitopes of HLA-DR in routinely fixed and processed tissues.[6,7] These antibodies offer the considerable advantage of allowing retrospective studies to be performed. The purpose of this investigation was to assess the potential value of the antibodies TAL-1B5 and LN3 in discriminating between benign naevi and malignant melanomas.

METHODS

Thirty-one naevocellular naevi of varying histological type, 25 cutaneous malignant melanomas and two metastatic malignant melanomas were retrieved from the files of the Leicester Royal Infirmary Department of Histopathology. Details of these cases are shown in Tables 1 and 2. All tissues had been fixed in 10% formol-saline and routinely paraffin wax-embedded.

Four micron sections were stained with the antibodies TAL-1B5 (Dako) and LN3 (Clonab). TAL-1B5[6] detects an epitope found on HLA-DR α-chains. LN3[7] recognizes a non-polymorphic epitope on the HLA-DR molecule. An avidin–biotin–alkaline phosphatase technique was employed throughout and both antibodies were used at a dilution of 1 in 20.

TABLE 1. Histological types of naevocellular naevi studied

Type	No
Compound naevus	9
Intradermal naevus	17
Blue naevus	2
Spitz naevus	2
Halo naevus	1

TABLE 2. Details of cases of malignant melanoma (MM) studied

Type	No.	TAL-1B5+	LN3+
Superficial spreading MM *in situ*	5	0/5	0/5
MM with dermal invasion < 0·76 mm	6	0/6	1/6
MM with dermal invasion 0·76–1·5 mm	9	3/9	3/9
MM with dermal invasion > 1·5 mm	5	3/5	3/5
Metastatic MM	2	0/2	0/2

Negative controls were performed by omission of the primary antisera. Tonsil fixed in the manner described above was used as a positive control tissue.

RESULTS

Twenty-eight of the 31 naevi showed no evidence of staining of naevus cells by either antibody. However, Langerhans cells, histiocytes and lymphoid cells provided a positive internal control in most sections. Two intradermal naevi contained only very occasional weakly LN3-reactive cells, and the halo naevus studied contained clusters of naevus cells showing LN3 reactivity on their surface although TAL-1B5 was negative. The positive cell clusters in the halo naevus were surrounded by an intense mononuclear infiltrate.

Details of the staining reactions of the cases of malignant melanoma are summarized in Table 2. In the positive cases staining was limited to a small proportion of the melanoma cells and never exceeded 25%. The staining appeared predominantly membranous although occasional cells showed cytoplasmic staining (Fig. 1). The positive cells tended to occur in the deeper parts of the lesions, often in areas with a prominent inflammatory infiltrate. Although no formal morphometric studies were performed it appeared that staining was more prominent in the larger tumour cells.

The two cases of metastatic malignant melanoma showed no evidence of TAL-1B5 or LN3 reactivity. In general TAL-1B5 staining was weaker than LN3 staining in melanocytic lesions and this may have been related to fixation, acidic fixatives being optimal for TAL-1B5 reactivity.

DISCUSSION

The first report of apparently anomalous HLA-DR expression was on cultured malignant melanoma cells.[1] Since then similar findings have been noted in tissue sections of melanomas[2-5] and other tumours including colorectal and breast carcinomas.[8,9] It has been suggested that HLA-DR expression may be related to the accumulation of mononuclear cells in these tumours.

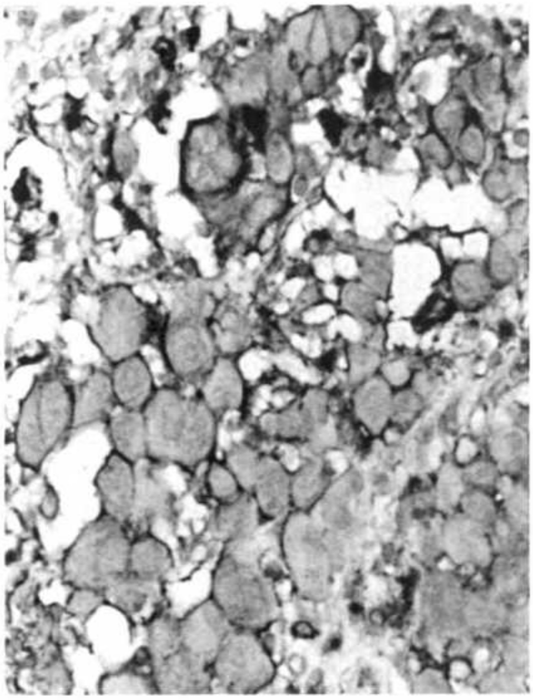

FIGURE 1. Malignant melanoma stained with LN3. There is surface staining of large melanoma cells (× 200).

Winchester *et al.*[1] found HLA-DR on six out of eight melanoma cell lines. However, the extent of reactivity varied markedly amongst the different cell lines. Nathali *et al.*[2] examined cell suspensions and cryostat sections from malignant melanomas and found heterogeneous HLA-DR expression. All their cases contained a variable proportion of negative cells. Ruiter and co-workers undertook two studies of HLA-DR expression in naevocellular naevi and malignant melanoma.[3,4] They showed that expression was absent from naevi but present to a varying degree in malignant melanoma. In one study[3] half of the primary cutaneous malignant melanomas showed heterogeneous positivity. However, in the other[4] only one out of eight primary cutaneous melanomas expressed HLA-DR. These results show that HLA-DR expression is a feature of some of the cells of some malignant melanomas but that there is considerable heterogeneity. Only seven (28%) of our 25 cutaneous melanomas were reactive with one or both antibodies. However, if one considers only invasive lesions this rises to seven out of 20 (35%) and if only lesions invading deeper than 0·76 mm are included, six out of 14 (42·8%) were positive. We have thus confirmed the heterogeneity of HLA-DR expression in malignant melanomas and the lack of reactivity of the vast majority of naevocellular naevi. Furthermore, our observation of increased staining in the deeper parts of deeper lesions is in accord with those of Ruiter *et al.*[3] Unlike that group, however, no staining was observed in metastatic malignant melanoma and we cannot concur that HLA-DR expression is necessarily a manifestation of progression in malignant melanoma.

This study has not attempted to examine the mechanisms of induction of HLA-DR expression on cells of melanocytic origin. HLA-DR expression can be induced in non-lymphoid cells as a result of stimulation with gamma-interferon.[10] This is produced by T lymphocytes[11] which are known to be abundant within the mononuclear infiltrate surrounding malignant melanomas and naevocellular naevi.[3] The function of HLA-DR when expressed in non-lymphoid cells is uncertain. It normally acts an antigen presenting molecule to T helper cells.[12] This may also be its function in non-lymphoid tissues and it could be responsible for the development of an immunological reaction by the host against tumour cells.

Our finding of HLA-DR expression on the naevus cells of a halo naevus is of interest with regard to a possible immunological reaction. Halo naevi represent a relatively unusual form of regressing naevocellular naevus in which a very intense mononuclear infiltrate is present. If not excised, regression will eventually occur.[13]

Our study indicates that HLA-DR is detectable in paraffin sections of malignant melanomas. However, the melanomas that were stained tend to be those that are more deeply invasive and we do not feel that any of them present a diagnostic problem. We therefore conclude that the antibodies TAL-1B5 and LN3 are not of value in the routine investigation of melanocytic proliferations. However, in view of recent findings that HLA-DR expression may be an adverse prognostic factor in malignant melanomas,[14] we feel that further studies of MHC class II products are justified.

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Failure of a helium-neon laser to affect components of wound healing *in vitro*

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SUMMARY

The red light of a helium-neon (He-Ne) laser has been reported to stimulate wound healing and cell growth. To investigate the nature of its influence on wound healing we have studied seven components of the healing process *in vitro*: human skin fibroblast, epithelial and endothelial cell proliferation, cellular migration from skin explants, collagen lattice contraction, collagen synthesis and glycosaminoglycans (GAG) secretion. We used a 5 mW He-Ne laser emitting a 1 mm diameter beam of wavelength 633 nm. Cellular proliferation was not affected by irradiation three times a day for 3 days. There was no effect on cellular migration or on the rate of collagen lattice contraction. The rate of collagen synthesis, measured as the incorporation of ^{3}H-proline into collagenase-sensitive protein, was no greater than that of controls and GAG secretion did not increase in the irradiated group. We have not found any significant effects of He-Ne irradiation.

Natural healing takes time, and humans quickly become impatient. As a result, open sores have been treated with medicaments and a range of natural and synthetic materials in an attempt to speed healing. Mester[1] advocated another approach, when he reported that low power helium-neon lasers could help healing in mechanically inflicted wounds, and later that they increased collagen production.[2] Since then, low energy lasers have been shown to have diverse effects on living tissues, effects known as laser biostimulation.[3] Augmentation of wound healing was confirmed by some,[4,5] but others have failed to find any improvement in wound or ulcer healing.[6,7,8] In some studies,[5] the effects of monochromatic, non-coherent red light were equal to those of laser light. *In vivo* studies have proved difficult to assess as so many local and systemic factors affect healing. Different workers have used different lasers each with its characteristic wavelength; exposure times and frequency of treatments have varied; irradiance has rarely been the same for two experiments. There is a wealth of data but comparisons of the results are difficult.

Rather than attempting to reproduce the conditions used in other experiments, it seemed more logical to employ a laser which is in routine clinical practice and assess its effects on cultured cells, following the instructions for normal clinical use.

METHODS

The laser was a 5 mW helium-neon (He-Ne) unit emitting a wavelength of 632·8 nm. The maximum beam diameter was 1 mm and its area 0·5 mm^2. The laser tube was fixed in a retort stand with the beam pointing down, so that it was possible to move the monolayer cell cultures, in their dishes, under the fixed beam. In effect this produced a brushing stroke of the laser beam over the cells, delivering approximately the same energy to all areas of the dish, i.e. 5 mJ/s distributed over 2 cm^2 or 10 cm^2 depending on the size of the dish. All the experiments were performed in a laminar flow cabinet which was cleaned with methanol before and after experiments, and sterilized with UVL when not in use. The energy delivered was expressed as average energy deposited per cm^2.

Cell cultures

Lines of fibroblasts (passage 6–10) from normal human skin were removed from liquid nitrogen storage and cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum, 4 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and bicarbonate (all from Gibco, Paisley, Scotland, U.K.) in 5% CO_2/95% air at 37°C. NCTC 2544 epithelial cells, originally from human skin,[9] were grown in the same medium. Endothelial cells, from human umbilical cords, were cultured in the same medium with endothelial growth factor and preservative-free heparin 50 U/ml. Routine cultures were made in Nunc plastic flasks (Gibco Ltd) and monolayers were dispersed with trypsin-versene for sub-culture or cell counts.

Proliferation assays

Replicate cultures were established in 24-well dishes (Day 0) and the medium was changed on Day 1. On Day 3 four wells were trypsinized and the suspensions were counted electronically (Coulter counter). A second group of four wells was left untreated while the remaining 16 wells were divided into four groups to receive varying amounts of He-Ne light. Irradiation was for 5, 10, 30 or 60 s three times daily for 3 days, giving an average energy deposited of 37·5, 75, 225 or 450 mJ/cm^2 per day. The medium was changed again on Days 3 and 4 and cell counts were made on all the wells on Day 6. Proliferation in controls between Days 3 and 6 was taken as 100% and that in irradiated cultures was expressed relative to controls; data were plotted as dose–response curves with time on the *x* axis. In early experiments the lids were removed from culture dishes prior to irradiation but later they were left in place, with no change in result. In some experiments the control cultures were made in a separate dish to exclude the possibility of stray irradiation: where this was done the controls were removed from the incubator for the same length of time as irradiated cultures but not transferred to the laminar flow hood.

Cell migration

A punch biopsy (4 mm) of normal human skin (13 year-old-female, upper arm) was chopped into 1 mm fragments and these were arranged in six-well dishes under coverslips in complete medium containing 20% fetal calf serum. The medium was changed every 4th day, and on the 3rd to 9th days explants in wells 1–3 were irradiated for 5 min each so that the energy delivered to each explant was 1·5 J/mm^2 per day for 7 days. On the 10th day the outgrowth from each explant was assessed by recording the number of epithelial sheets (very large sheets were counted as two small sheets), and counting individual fibroblasts up to a maximum of 500. Fibroblast outgrowth was given a score of 0–3 with 0 for zero growth, 1 for 0–50 cells, 2 for 51–500 cells and 3 for over 500. The number of epithelial patches was added to the fibroblast score to give a total score for each explant and a mean score was calculated for the 10 irradiated explants and the 12 non-irradiated explants.

Collagen lattices

Lattices of Type I collagen, 35 mm in diameter and containing approximately 2 × 10^5 normal skin fibroblasts were prepared in bacteriological grade six-well dishes.[10] Individual lattices were irradiated for 5 or 10 min, three times daily on Days 1, 2 and 3 giving average energy deposition of 450 or 900 mJ/cm^2 per day for 3 days. Lattice diameters were measured by placing the dishes on mm^2 graph paper over a light box and recording the mean of two diameters for each lattice.

Collagen synthesis

Collagen synthesis by confluent monolayer fibroblast cultures was assessed as incorporation of ^{3}H-proline into collagenase-sensitive protein, using collagenase Type III (Advance Biofactures Corporation, Lynbrook, NY, U.S.A.).[11] Cultures in 35 mm diameter wells were irradiated twice daily for 3 days for up to 2·5 min each giving a maximum average energy deposited of 150 mJ/cm^2 per day. The ^{3}H-proline was added on the 3rd day of irradiation for a further 24 h. Non-radioactive cultures were subjected to the same irradiation and provided cell counts for calculation of collagen and other protein synthesis as d.p.m. per 10^5 cells.

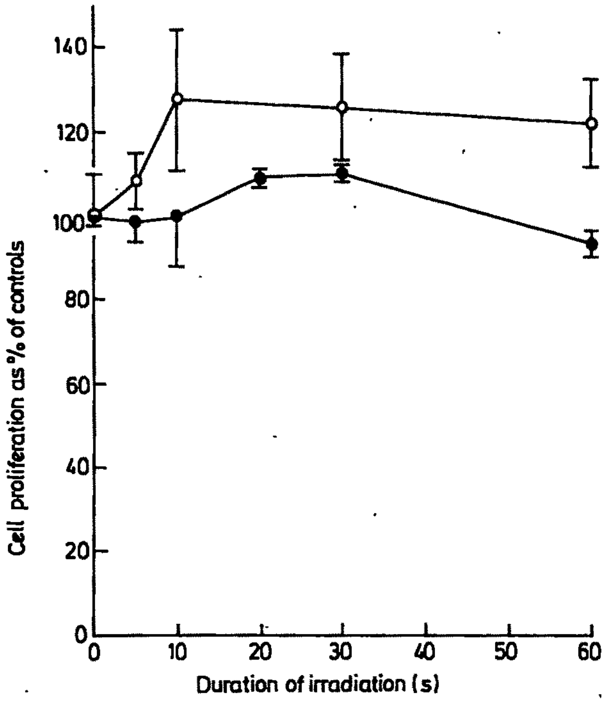

FIGURE 1. Fibroblast proliferation in two experiments, shown for different irradiation times. Values are means (± SEM) from four replicate cultures. Each exposure was repeated three times daily for 3 days.

Glycosaminoglycans secretion

Fibroblast monolayers were set up in six-well dishes and irradiated twice daily for 3 days for 2 or 5 min per well as the cells approached confluence: this gave an energy deposited of 120 and 300 mJ/cm^2 per day. The medium was then removed and processed while the cells were trypsinized for counting. Media were dialysed, concentrated, digested with hyaluronidase and treated with trichloracetic acid (final concentration 5%) to remove protein.[11] Uronic acid contents in the supernatants were measured colorimetrically with *m*-hydroxydiphenyl[12] and the glycosaminoglycans (GAG) secretion was expressed as μg uronic acid per 10^5 cells.

RESULTS

Cell proliferation

Figure 1 shows the results of two experiments designed to assess the effect of irradiation on fibroblast proliferation. In one there was a general increase in proliferation (open circles), unrelated to length of irradiation, reaching a maximum of 24% in the 10 s exposure group, where it just attained statistical significance. There was more variation in the second experiment (closed circles) and none of the values were significantly different.

Effects on NCTC 2544 epithelial cell proliferation are shown in Figure 2. Again there was a general increase in proliferation in one experiment (open circles) but without a dose–response effect and never reaching statistical significance. Values in the second experiment were within ± 10% of controls.

In the first experiment with endothelial cells, treated cultures proliferated less than controls. In a second experiment there was no net proliferation in untreated controls between Days 3 and

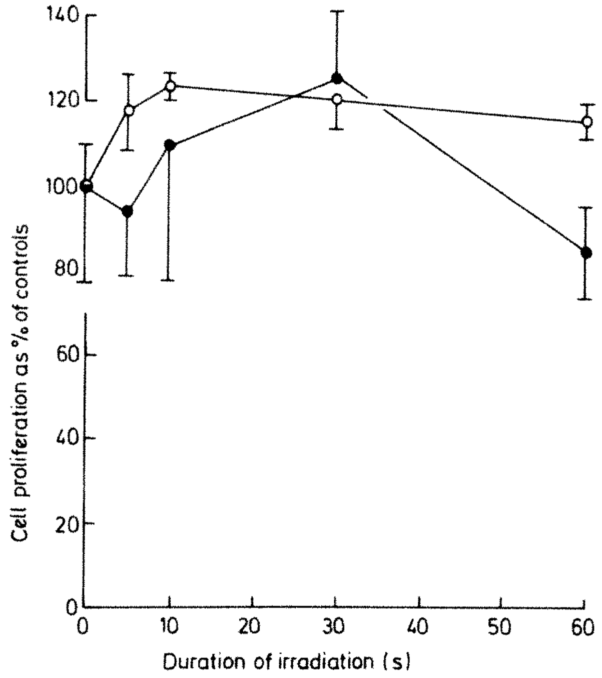

FIGURE 2. Epithelial cell proliferation in two experiments, shown for different irradiation times. Values are means (± SEM) from four replicate cultures. Each exposure was repeated three times daily for 3 days.

TABLE 1. Effect of He-Ne irradiation on cell migration from skin explants

Group	Explants (*n*)	Epithelial growth	Fibroblasts	Overall mean score (± SEM)
He-Ne	10	7	12	1·90 ± 0·46
Control	12	9	14	1·92 ± 0·41

6: the relative increases of 4% and 8% in irradiated cultures were not considered to be significant.

Cell migration

Scores for mean migration per explant are shown in Table 1. These include both epithelial and fibroblast components and were judged on the 10th day of culture when the fibroblasts were still sparse enough to count. Clearly, the scores of the irradiated and control groups are identical. An earlier experiment, where the migration was assessed visually without cell counts, supported this.

Collagen lattice contraction

Figure 3 shows that irradiation had no effect on the rate of contraction of fibroblast-populated collagen lattices and a second experiment gave the same result.

Collagen synthesis

In Table 2 mean values for collagen synthesis in irradiated cultures are within 6% of controls and values for other protein synthesis are even more consistent. In a second experiment where technical problems with the collagenase invalidated the collagen data, mean values for total

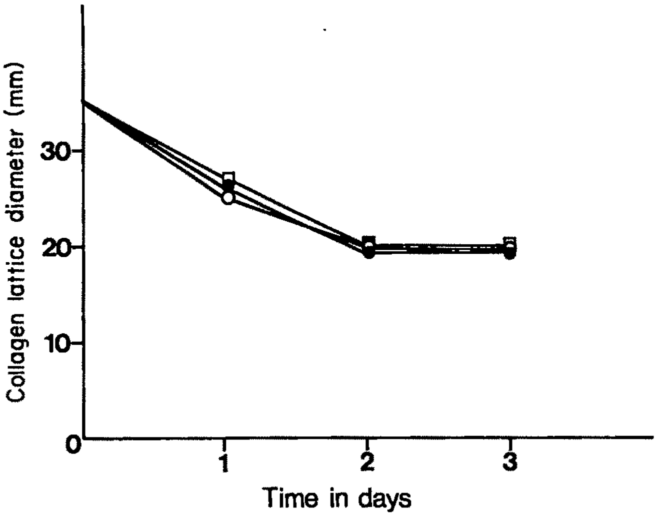

FIGURE 3. Contraction rates for control (○) and irradiated collagen lattices: 5 min (●) and 10 min (□) irradiation values are means from four lattices: standard errors were all less than ± 1 mm.

TABLE 2. Collagen synthesis in He-Ne irradiated skin fibroblasts (mean d.p.m., $n=3$)

Group	Collagen/10^5 cells	Other protein/10^5 cells	RCS
Controls	24306 ± 944 (SEM)	122328 ± 1913	3·6 ± 0·2
× 1 min	25103 ± 1006	121203 ± 11537	3·7 ± 0·3
× 2·5 min	23931 ± 2419	122898 ± 9552	3·4 ± 0·7

RCS = relative collagen synthesis.

TABLE 3. Effect of He-Ne irradiation on fibroblast GAG secretion

Cell line	Group	Cells (10^5)	GAG output per 10^5 cells (μg uronic acid)	% change
HSF 22	Control	5·57 ± 0·03*	16·6 ± 1·6	
	2 min	5·35 ± 0·07	14·6 ± 2·6	−12
	5 min	5·05 ± 0·12	18·5 ± 2·6	+12
HSF 36	Control	4·04 ± 0·08	29·6 ± 2·4	
	2 min	4·14 ± 0·06	24·8 ± 3·4	−16
	5 min	4·05 ± 0·03	27·7 ± 3·9	−6

* Mean values are shown with standard errors; $n=5$.

protein synthesis were 135393 ± 7200 d.p.m./10^5 cells for the controls, and 135781 ± 1867 and 125971 ± 1667 d.p.m./10^5 cells (± SEM; $n=5$) with 0·1 and 2·5 min irradiation. The 7% depression in total incorporation in the longest-irradiated (2·5 min) cultures was statistically significant (t-test; $P < 0·01$).

GAG secretion

The results of two experiments (Table 3) both showed unchanged rates of secretion after irradiation. None of the small differences reach statistical significance. Data from cell counts are included because secretion per cell is inversely proportional to cell density.[13] The very similar densities therefore validate the comparison of secretion and also serve to support the lack of effect on fibroblast proliferation reported in Figures 1 and 2.

DISCUSSION

Our experiments have failed to demonstrate any biological effect of He-Ne laser light at energy intensities suitable for clinical use: the changes recorded after irradiation were all small, sometimes negative and rarely statistically significant. This lack of effect could be explained in any of four ways: insensitivity in our systems; the selection of inappropriate models; deposition of inadequate energy; or a true absence of biological effect on wound healing. The first possibility is unlikely. The cellular proliferation model is a reliable and sensitive measure of both stimulatory and inhibitory influences;[14] incorporation of ^{3}H-proline into collagenase-labile protein is a sensitive measure of collagen production[11,15] and GAG measurements are highly reproducible. Lattice contraction and the migration of cells from explants are less sensitive tests and are best at detecting inhibitory influences.[16]

The second explanation might be that our models were not the right ones to investigate the biological effects of a He-Ne laser. No laboratory study can mimic the complex influences and interactions of wound healing; the living skin equivalent[17] has its drawbacks and does not include a wound equivalent. We opted to study a range of relevant cellular functions, accepting that we could not take into account circulating mediators or cellular interactions which might act as promoting factors.

This study was not designed to confirm previous results using similar models. Indeed, the variation in methodology and dosimetry of published papers is enormous. The aim of the present study was to test a therapeutic clinical laser for *in vitro* effects. The fact that it was a narrow beam clinical laser explains the problems in expressing the dose. The only helpful way is an average energy deposited per cm^2 cells. Other workers have used divergent lasers and mirror systems to spread out a narrow beam and make it cover the experimental field. This manoeuvre does not mimic the clinical setting because the electromagnetic characteristics of an intense narrow beam differ from those of a divergent beam. For our cellular proliferation experiments the energy delivered to the cells was between 37·5 and 450 mJ/cm^2 per day. In a comparable experiment Boulton and Marshall[18] used 90–180 mJ/cm^2 per day and detected increased cellular proliferation. Some workers have reported faster wound closure[19] and repair of soft tissue injuries[20] using doses similar to our own, while others have used much higher doses to produce a clinical effect. Kana *et al.*[5] found that 4 J/cm^2 daily for 9 days was optimal to produce an increase in the rate of wound closure and collagen formation. Surinchak[7] used 4·2 J/cm^2 for 14 days on incisions in rat skin to show a temporary 55% increase in breaking strength.

Lastly, we must consider whether low-dose lasers do have any biological effects. At high energy levels they may have thermal effects: some of the changes found by Kana *et al*,[5] for instance, also occurred with coherent red light. At low levels the effects are photobiological.[3] Many have investigated the biological effects on animal and plant tissues.[21] Some of the effects reported seem contradictory. In the neonatal chick, low dose radiation inhibited cell division in the lens[22] but increased mitosis in the retinal pigment epithelial cell.[23] Furthermore, He-Ne irradiation (2·4 J/cm^2 per day) inhibited angiogenesis in the chick chorioallantoic membrane (Cherry, personal communication) but this effect could not be harnessed to help psoriasis.[24]

More fibroblasts were seen in the bed of healing wounds after He-Ne irradiation.[25] Foreskin and human skin fibroblasts were cultured by Boulton and Marshall[18] and exposed to 90 mJ/cm^2 of coherent light at 633 nm on one or two occasions. Cell numbers increased significantly when compared with non-irradiated controls; incoherent light had no effect. Enhanced collagen synthesis by fibroblasts has also been demonstrated[26] and this again contrasts with our own findings. The figures reveal, however, that cell lines producing collagen in the normal range were little affected by He-Ne, while those with exceptionally low basal production could be stimulated many fold.

In conclusion, we have studied the effects of He-Ne laser irradiation on the proliferation of cultured cells and on several aspects of their metabolism and have been unable to detect any significant positive effect. We cannot explain the reported benefits of He-Ne irradiation on wound healing.

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agar in the Department of Microbial Diseases, The Institute of Dermatology, Lisle Street, London, U.K. The diagnosis of scabies was confirmed by direct microscopy of the contents of 'burrows' removed on the point of a fine sterile needle. Swabs for bacterial culture were taken from selected patients, placed in transport medium and transferred to the laboratory within 4 days. They were cultured subsequently in the Department of Microbial Diseases, the Institute of Dermatology in London.

In selected patients the diagnosis of skin lesions was confirmed by biopsy under local anaesthesia with 2% xylocaine using 4 mm diameter disposable biopsy punches (Stiefel Laboratories, Slough, U.K.). Skin was fixed in formol saline or Bouin's fixative and subsequently paraffin-embedded. Sections were stained with haematoxylin/eosin as well as special stains such as Giemsa and periodic acid Schiff where appropriate.

Diagnostic skin snips for the isolation of *O. volvulus* microfilariae had been taken 3 months earlier from over the iliac crest of most of the patients included in this survey using a technique described previously.[7] Quantitative microfilarial loads were calculated for each sample, although in a few instances only the presence of parasites was noted. Skin snips were not taken from children below the age of 4. These results were compared with the dermatological findings after the survey had been completed.

The results of the survey were analysed statistically using the chi-squared test.

RESULTS

Zapallo Grande

A total of 106 patients (59 Amerindian, 47 Negroid) were examined in detail. Their ages ranged from 6 months to 63 years (mean 25 years). In these individuals 229 different morphological patterns of skin disease were seen, 123 of which were thought to be related directly to onchocerciasis. Diagnotic skin snips were not taken from eleven children in this sample; in addition six patients (all Negroid) were negative for microfilariae on skin snipping.

The results of this survey are seen in Tables 1 and 2. The prevalence of skin disease, not directly attributable to onchocerciasis is seen in Table 1. It was found that the commonest skin conditions were scabies, pityriasis versicolor, pyoderma, insect bites and dermatophytosis.

TABLE 1. Skin lesions not due to onchodermatitis in Zapallo Grande

Ethnic group	PV*	Dermatophyte	Scabies	Pyoderma	Insect bites	Others†	Total
Amerindian	4	6	18	10	6	5	59
Negro	11	1	5	4	0	17	47
Ratio Amer:Negro	0·4‡	6	3·6‡	2·5	—	0·3	1·3

* PV, pityriasis versicolor.

† Molluscum contagiosum 1, seb. dermatitis 2, hand eczema 1, miliaria rubra 2, annular erythema 2, viral warts 2, epidermal naevus 1, acne vulgaris 2, paronychia 1, cutaneous leishmaniasis 1, palmar/plantar keratoderma 2, malnutrition 1, lichen spinulosus 1, urticaria 1, keratoacanthoma 1. In addition one patient had an unclassified papular dermatitis associated with a lymphocytic infiltrate of the skin.

‡ Significant ($P =$ or $< 0·05$).

TABLE 2. Skin changes *associated with onchocerciasis in Zapallo Grande

Ethnic origin	Acute papules	Gluteal dermatitis	Gluteal atrophy	Gen atrophy	Sowda	Hypo-pigment	Aneto-derma	Nil	All
Amerindian	19	17	2	12	0	3	1	3	52
Negro	7	11	6	1	2	0	0	11	37
Ratio: Amer:Negro	2·7	1·6	0·3	12†	—	—	—	0·25†	1·4

* Patterns of skin change observed: acute papular dermatitis, gluteal dermatitis (chronic papular dermatitis), gluteal atrophy, generalized atrophy, Sowda, hypopigmentation, anetoderma, no abnormality (nil).

† Significant ($P < 0{\cdot}05$).

There were no significant differences in the prevalence of these conditions in patients from different ethnic groups apart from pityriasis versicolor which was commoner in those of negro extraction ($P < 0{\cdot}005$). Other conditions are listed in the Table. Some less common conditions were also seen and their diagnosis was confirmed where necessary by biopsy.

In addition a number of patients had skin changes directly related to onchocerciasis. A total of 30 (34%) patients out of 89 with known microfilarial loads were found to have typical onchocercal nodules. These were commonly found over the iliac crest, the apex of the natal cleft (Fig. 1) or on the scalp. The commonest skin abnormality judged to be related to the infection was the presence of multiple lichenified papules, hyperpigmentation, scars and excoriations over the sacral and gluteal regions (Fig. 2). These changes were seen in 28 patients. In eight additional patients cutaneous findings were limited to atrophy and subsequent loss of elasticity of the skin in this area. The latter changes were more common in Negroes than Amerindians but the difference did not achieve statistical significance. The next most frequently observed change

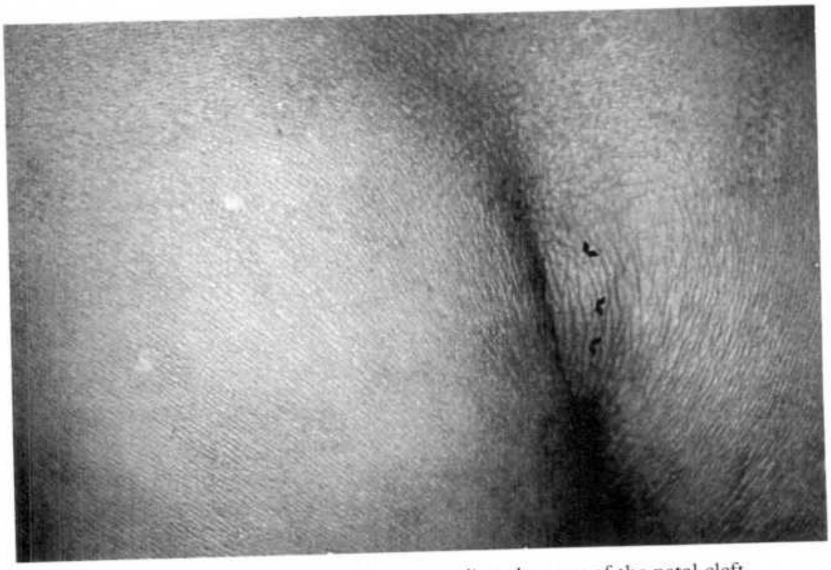

FIGURE 1. Onchocercal nodule (arrowed) at the apex of the natal cleft.

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Onchodermatitis—correlation between skin disease and parasitic load in an endemic focus in Ecuador

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SUMMARY

Onchocerciasis is a chronic parasitic infection in which infiltration of the skin by microfilariae has been associated with a number of different pathological changes. This survey compared the prevalence of different forms of skin disease in two villages, one of which was located within the endemic zone for onchocerciasis (Zapallo Grande), in a lowland rain forest area of western Ecuador. The commonest skin diseases in both villages were scabies, pyoderma and pityriasis versicolor. In addition changes closely correlated with the presence of microfilariae in skin snips were found in Zapallo Grande—such as atrophic gluteal changes, and acute and chronic papular dermatitis. The only other skin disease associated with onchocerciasis was widespread tinea corporis due to *T. rubrum*. The Amerindians in the endemic onchocerciasis area were more likely than Negroes to have generalized atrophic changes of the skin, whereas in the latter group significant numbers of individuals had no obvious skin lesions but large numbers of microfilariae were detected in skin snips. Acute papular dermatitis was common in both groups and in biopsied lesions microfilariae could usually be identified within the epidermis or close to the dermo-epidermal junction. One patient had developed severe reactive onchodermatitis (Sowda). Swabs taken from onchocercal skin lesions showed no evidence of skin surface carriage of *Staphylococcus aureus*. Changes classically associated with onchocerciasis such as pretibial hypopigmentation (leopard skin) and hanging groin were notably absent in this population.

Onchocerciasis is a chronic infection of humans affecting the skin and eyes caused by *Onchocerca volvulus*, a filarial worm transmitted from man to man by blackflies of the genus *Simulium*. This

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disease is confined to the African continent, parts of Central and South America and the Arabian peninsula, yet in these areas it may affect up to 50 million people.[1] Adult worms are present in large palpable subcutaneous nodules, but superficial tissues are diffusely infiltrated with microfilariae. The presence of the latter has been associated with a number of different clinical lesions in both the eye and the skin.

Lesions of the skin ascribed to onchocerciasis encompass both acute and chronic forms.[2] Acute changes include diffuse cutaneous infiltration and oedema accompanied by itching. The chronic features range from asymmetric lichenified onchodermatitis accompanied by severe itching (Sowda) to pretibial hypopigmentation (leopard skin) and premature ageing of the skin with acquired ichthyosis. However, the relationship between the presence of microfilariae and the development of skin changes is difficult to predict, with some highly symptomatic lesions containing few organisms and, at the other extreme, areas of clinically normal skin infiltrated with large numbers of microfilariae.[2,3] Clinical changes occur at a variable, and, frequently, long interval after the initial infection. In addition in endemic areas where the majority of the population harbour a pathologically significant microfilarial load, other skin diseases coexist with onchocerciasis. It is important to define which changes are due to onchocerciasis and whether the presence of microfilariae in the skin influences the appearance or course of other dermatoses.

We have investigated the prevalence of skin diseases in a recently described focus of onchocerciasis in Ecuador and compared the different skin lesions found in villages in hyper- and hypoendemic locations. The disease was first reported in Ecuador in 1980[4] and a major focus of infection in the province of Esmeraldas has been described subsequently.[5] The *Simulium* vectors of the disease are found in this region,[6] which was the location of our investigation.

METHODS

Patients

Patients were seen in one of two sites, Zapallo Grande or San Jose, which are both villages on the Rio Cayapa in Esmeraldas Province in coastal Ecuador and only accessible by river. The inhabitants of these areas belong to two main racial groups, Negroid or Amerindian (Cayapa tribe), there being almost no intermarriage between the two groups. At Zapallo most of those seen originated from adjacent villages, particularly those further upstream; San Jose is 20 km downstream from Zapallo. People of both ethnic groups were seen at Zapallo although Amerindians predominated; whereas all the individuals examined in San Jose were Negroid.

All those attending the clinics had a general medical examination and personal details were entered on a questionnaire form. The main purpose of the study was to determine the prevalence of skin disease and hence the eyes were not examined in detail, although a severe degree of ocular onchocerciasis exists in this population. Apart from a routine dermatological examination all patients were examined for evidence of skin lesions ascribed previously to onchocerciasis.[3] These included the presence of itchy papules and micropapules, atrophy over the buttocks or other sites, asymmetric lichenification on the limbs, leucoderma and subcutaneous nodules. None of the patients studied had received treatment with either diethylcarbamazine (DEC) or ivermectin.

Investigations

Skin scrapings were taken from patients with suspected mycoses and examined by direct microscopy in 30% potassium hydroxide. Further samples were also cultured on Sabouraud's

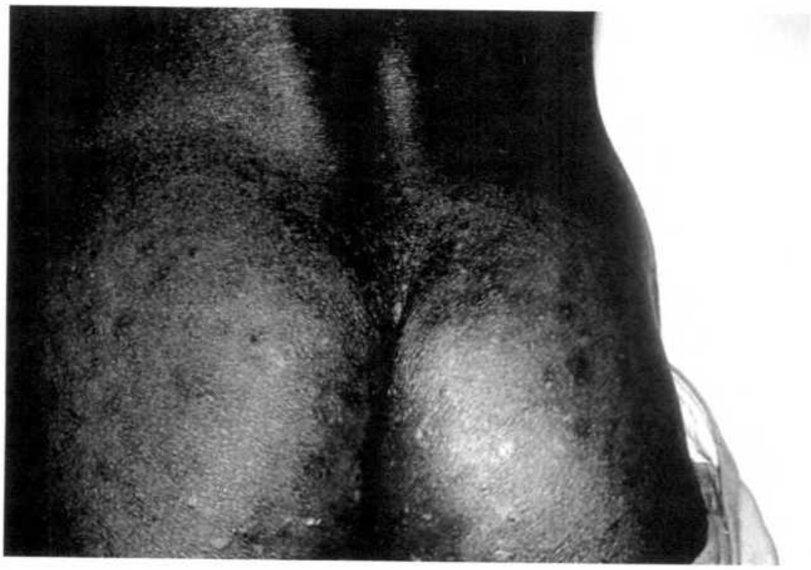

FIGURE 2. Onchocerciasis—gluteal scars and papules.

as a rash consisting of sparsely distributed papules (Fig. 3), some of which appeared pustular. hese were itchy and were seen mostly over the arms and upper back. They were accompanied excoriations and in some patients by atrophic hypopigmented scars. The skin also appeared ghtly oedematous. The papules could be distinguished from scabies and *Pityrosporum* lliculitis by direct microscopy. In three patients biopsies of these lesions showed intraepideral microabscesses or subepidermal microabscesses containing eosinophils and neutrophils nongst which, in some cases, the remains of microfilariae could be identified (see below). In

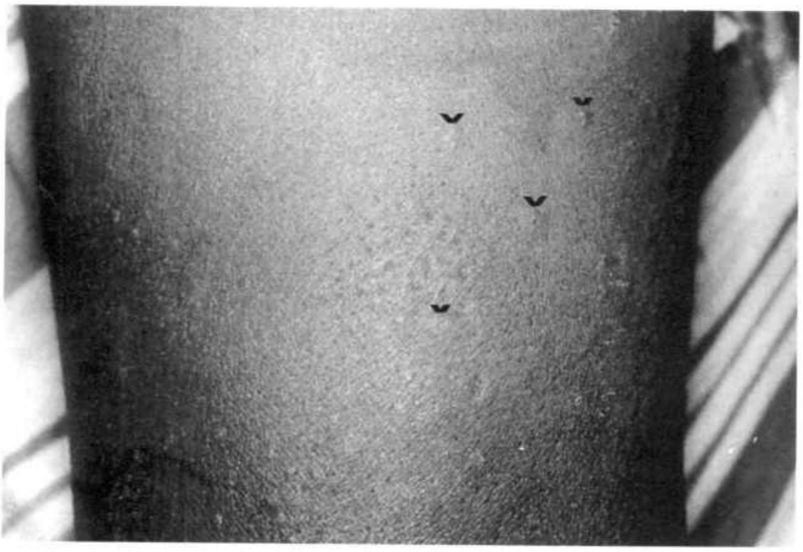

FIGURE 3. Acute papular dermatitis (arrowed) in onchocerciasis on the back of the forearm.

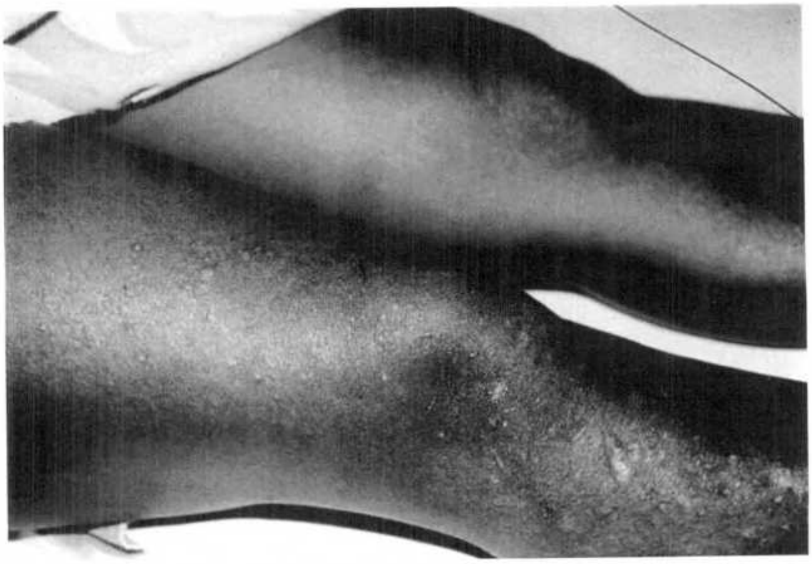

FIGURE 4. Reactive onchocerciasis or Sowda affecting the right leg.

addition to these changes it was noticeable that affected Amerindians were more likely to show marked atrophic changes of the skin with loss of elasticity ($P<0{\cdot}01$). Twelve Amerindian patients had marked atrophic changes and some reported poor wound healing and prolonged bleeding in response to injury.

In three younger Amerindian patients (all below the age of 21) numbers of hypopigmented scars were seen in addition to changes around the gluteal area. One patients showed numerous hypopigmented atrophic macules, suggestive of an anetoderma. All these latter patients had microfilarial loads in excess of 20 per mg of skin. Other changes included one Negroid patient with unilateral lichenification associated with intolerable itching affecting the right leg and inguinal lymphadenopathy, Sowda (Fig. 4). This girl also had papular changes on her shoulders. Some patients (14) had no discernable skin changes despite the presence of microfilariae. This was mainly seen in Negroid patients ($P<0{\cdot}005$). Only one patient in this survey had pretibial macular hypopigmentation or 'leopard skin', often described in African cases of onchocerciasis, and no microfilariae were detected in skin snips.

San Jose

Sixty-nine patients (36 male, 33 female) were examined in San Jose. The sample was unselected and those seen ranged in age from six months to 68 years (mean 28 years); 29 were below the age of 15. Apart from two patients with atrophic changes in the gluteal region, there were no patients with skin lesions suggestive of onchocerciasis. In particular there were no patients with nodules. The distribution of disease is seen in Table 3. The commonest diseases were pityriasis versicolor, pyoderma and scabies. Only five patients had microfilarial loads and these included the two with onchocercal changes in the gluteal region. The loads were 21, 12, 5, 3 and 2 microfilariae per mg of skin respectively. Once again the patients with scabies were mainly children or infants and all but one appeared to have to have secondary bacterial infections. Sites of secondary infection included the fingers, waist and scalp. One patient with scaling on the palms and onycholysis was found to have an infection caused by *Hendersonula toruloidea*.

TABLE 3. Skin disease in San Jose

	PV*	Scabies	Pyoderma	Onchodermatitis	Dermatophyte	Other skin diseases
Numbers affected	12	9	11	2†	6	23‡

* PV, pityriasis versicolor.

† Atrophic gluteal changes.

‡ Eczema 2, onychomycosis due to *Hendersonula toruloidea* 1, acne vulgaris 2, pruritus 1, candidosis (mouth) 1, paronychia 2, seborrhoeic dermatitis 1, keratoderma 2, molluscum contagiosum 1, herpes zoster 1, viral warts 3, lichen simplex 1, insect bites 3, ichthyosis 2.

Trichophyton rubrum was isolated from the patient with tinea cruris. Only two patients had acne vulgaris and in both cases the main changes were seen on the forehead. One patient had keloidal folliculitis of the scalp.

Correlation of skin disease with the presence of dermal microfilaria

An attempt was made to correlate the presence of microfilariae in the skin with the appearance of different skin diseases in the two areas surveyed. The results are shown in Table 4. Only those skin lesions seen in more than five patients were analysed. There is a clear correlation between the presence of microfilariae in the skin and papules, generalised or atrophic changes localized in the buttock region as well as the finding of nodules ($P < 0{\cdot}005$). However, all the other conditions appeared equally in both groups apart from pyoderma which was commoner in the patients without microfilariae ($P < 0{\cdot}005$). This result may be explained by the prevalence of bacterial infection in infants in whom skin snips were either negative or not assessed. Four of the five patients with tinea corporis due to *T. rubrum* in whom microfilarial counts were assessed had

TABLE 4. Correlation between skin disease and microfilarial load in Zapallo Grande and San Jose, Esmeraldas

Microfilariae (per mg)	Skin changes							
	Gluteal change	Atrophy*	Acute papules	Nodules	Scabies	Dermatophyte	Pyoderma†	P.V.
> 50	6	1	3	2	3	2‡	1	1
20–50	14	3	5	5	7	2‡	1	2
10–20	5	4	4	3	3	0	2	3
0–10	8	1	4	7	4	1	2	2
Positive only	4	1	2	2	1	1	0	4
Total positive	37§	10§	18§	19§	18	6§	6	12
Not done	6	0	2	0	4	3	5	3
Negative	0	0	0	1	13	3(1‡)	17	19

* Generalized or localized atrophy, e.g. gluteal area.

† Includes impetigo and sores.

‡ Tinea corporis.

§ Significantly associated with presence of microfilariae.

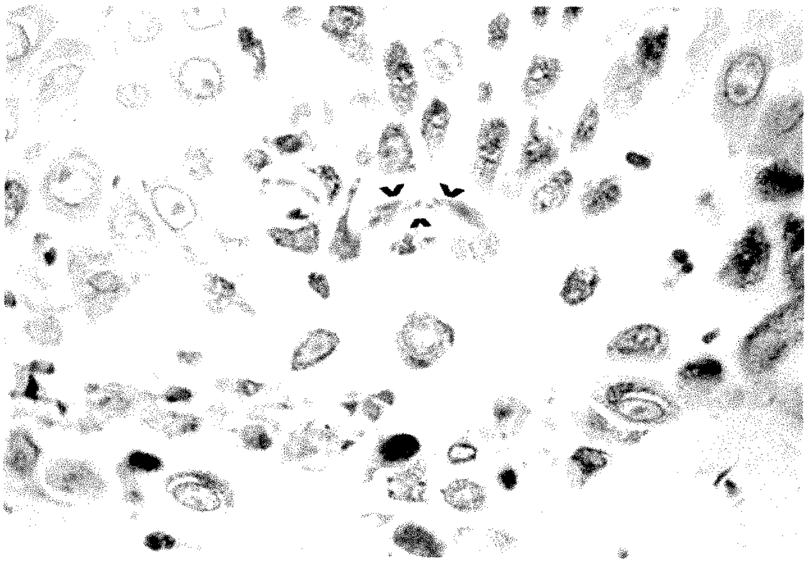

FIGURE 5. Biopsy from an acute papular lesion (cf. Fig. 4.). There is a suprabasal cleft and acantholysis. The remnants of a microfilaria can be seen (arrowed). H & E × 640.

parasitic loads of over 20 microfilariae/mg and this infection was significantly associated with the presence of microfilariae ($P < 0{\cdot}005$). The lesions of tinea corporis were mainly found across the shoulders or around the buttocks, areas frequently affected by onchodermatitis.

Histopathology

Fourteen punch biopsies were taken from patients with skin conditions identified as being either due to onchocerciasis or insect bites. The tissue changes were all within the range of alterations which would be expected for onchocerciasis with infiltrates ranging from mild (three samples) to severe (11) perivascular accumulations of lymphocytes, plasma cells and other mononuclear cells. In two of three samples from patients with papular changes there were foci of eosinophils and macrophages associated with degenerating microfilariae in the epidermis (Fig. 5) and upper dermis close to the basement membrane. Microfilariae were present in nine of the 14 samples and those where microfilariae were not detected nevertheless had changes in keeping with parasitic disease. An increase in the general level of dermal eosinophils were seen in two biopsies of papular dermatitis. In both instances the patients were thought to be affected by severe insect bites.

Bacterial cultures

Swabs were taken from the anterior nares in 29 patients and six (21%) were positive for *Staphylococcus aureus*. In one individual *Klebsiella rhinoscleromatis*, associated with the disease rhinoscleroma, was isolated but there were no clinical abnormalities. No other pathogens were identified, the other bacteria cultured from this site being mainly micrococci or coryneforms. There was no evidence of carriage of *S. aureus* on onchocercal dermatitis lesions in 12 patients sampled. It is of interest that five of the *S. aureus* isolates were resistant to penicillin and three to tetracyclines. *S. aureus* was isolated from three of eight lesions of pyoderma sampled.

β haemolytic streptococci (Group A) were only found in two of these lesions, in one case together with *S. aureus*.

DISCUSSION

Recent work has established that the area of study encompasses an endemic focus of onchocerciasis;[5,6] Zapallo Grande, one of the villages is within the endemic area as are adjacent communities upstream, whereas San Jose, the other, is outside this area. Individuals travelling from the latter site, however, are at risk from exposure to the disease and the climate and socioeconomic conditions are identical in both villages. The population have an adequate diet of fish, vegetables and fruit, the main occupation being farming or forestry. Malaria is endemic throughout the locality.

The survey showed a difference in the patterns of skin disease found within the two communities. In Zapallo significant numbers of patients had onchocercal nodules and severe or localized atrophic changes as well as pruriginous areas of papular and lichenified skin particularly around the buttocks. One patient showed an asymmetrical rash consisting of hyperpigmentation and lichenification on the left leg associated with inguinal lymphadenopathy, a pattern compatible with reactive onchocercal dermatitis (Sowda), a form of the disease orginally associated with a focus in North Yemen[8] but now known to occur elsewhere e.g. West Africa, Sudan.[9] A case has recently been described in Ecuador.[10] In the same village a number of patients had diffuse micropapules and pustules on the skin associated with pruritus. All these morphological forms correlated with the presence of microfilariae in skin snips and were absent in patients without microfilariae seen in San Jose, and are probably directly related to onchocerciasis. The last form of skin disease associated with onchocerciasis, papular, is similar to that seen with multiple insect bites. However the monomorphic nature of the lesions and the presence of microfilariae in dermal or epidermal microabscesses[11] in biopsy specimens suggests that the rash is due to onchocerciasis, although it does not exclude the possibility that biting insects, including *Simulium* species, contribute to the tissue changes.

The prevalence of other common diseases seen, including scabies and pyoderma, was similar in both areas. Pityriasis versicolor was commoner in San Jose. However it was less common in Amerindians who accounted for a substantial proportion of the sample in Zapallo, the difference possibly reflecting a difference in ethnic susceptibility. Tinea corporis across the shoulders or around the buttocks was seen only in patients with substantial microfilarial loads. This form of infection was confined to Amerindians and it is not clear whether it represents an increased susceptibility to dermatophytosis associated with onchocercal infection or whether ethnic or genetic susceptibility factors are involved. However, as it was not seen in Amerindians with no or low microfilarial loads the former is more likely. It has been reported that the prevalence of lepromatous compared to tuberculoid leprosy is higher in patients with onchocerciasis, suggesting that immunosuppression associated with the filarial disease may predispose to certain other types of infection.[12] Tinea corporis may be an example of this phenomenon.

The main clinical features of onchodermatitis have been described before.[13,14] The chronic changes of the disease include atrophy, pigmentary anomalies and hanging groin. In this sample there were no patients with hanging groin which is presumed to result from atrophic changes in the inguinal area combined with enlargement of inguinal lymph nodes with lymphoedema. The only patient with 'leopard skin'[15] changes, which are a combination of spotty hypo-and hyperpigmentation combined with atrophy interspersed with lichenification usually on the shins, lived in the non-endemic area, San Jose, and had no microfilariae on skin snips. While this does not exclude onchocerciasis as the cause, it does emphasise that this particular clinical pattern is associated with lichenification and chronic scratching of different aetiologies. The rarer pattern of long standing onchodermatitis associated with active killing of microfilariae in the skin, Sowda, was seen in a single patient who was not free from skin lesions in other sites. For instance she had papules on the forearms and shoulders as well as one palpable nodule. The reasons why this form should be uncommon in the area is not known. However, the biting frequency of flies or inherent variations in the development of resistance may affect the development of reactive onchodermatitis.

The acute forms of onchocerciasis are more difficult to recognize. For instance, in children diffuse infiltration with obliteration of normal skin marking may occur. The acute papular lesions may be seen in individuals of any age. However, in some patients it may be difficult to separate the appearance of papules from insect bites. The clinical diagnosis of insect bites was made in a number of patients although this is difficult to prove without biopsy evidence. Three patients with the papular pattern were biopsied and showed microfilariae in the dermis as well as subepidermal or intraepidermal microabscess formation. In two of these, remnants of microfilariae could be seen. The reaction may, therefore, represent killing of parasites within or adjacent to the epidermis. The histology is similar to that seen with the Mazotti reaction[16] which occurs in patients given microfilaricidal drugs, typically diethylcarbamazine. The reason it was more common in Amerindians is not known. Conversely atrophic changes were mainly seen in Negroes. Ethnic factors may also play a part in determining disease patterns although other factors are potentially involved. For instance the Amerindians seldom have walls on their houses and in the case of the women usually wear nothing above the waist. Exposure to the *Simulium* vectors in the patients of different racial groups living in the same area may be different. Although it is difficult to prove, there is strong circumstantial evidence to suggest that excoriation of these papules produces the chronic papular dermatitis which is most prominent in sites where there is likely to be a high microfilarial density such as the buttocks. Finally it is important to note that a substantial proportion of patients had high microfilarial counts but no evidence of skin disease or diffuse or localized skin atrophy presenting with shiny wrinkled skin and increased fragility.

The pattern of the other forms of skin disease is similar to that reported in other rural tropical areas. One striking finding was the almost total lack of eczema. There are a number of community based surveys of skin disease in the humid tropics with which these findings can be compared. For instance Bechelli and co-workers in Brazil found that 90% of the population surveyed had skin disease, the commonest being pediculosis, pityriasis versicolor, pyoderma, acne and dermatophytosis.[17] The commonest non-infective conditions were naevi and pityriasis alba seen in 16·8 and 9·9% of the population respectively. In a survey based on children presenting for treatment of skin conditions in South Africa[18] the common dermatoses were eczema (18·7%), pyoderma (8·3%), dermatophytosis (7·5%) and seborrhoeic dermatitis (3·4). By contrast, in Hong Kong[19] a mixed aged population coming for skin treatment had eczema (31–51%) dermatophytes (11·4%) and viral infections (4·8%) as the commonest conditions. These studies emphasize the difference in tropical populations where climate for instance may increase the prevalence of bacterial infections through humidity and temperature.[20] There are also differences seen in these surveys between populations in the urban versus rural tropics, with a predominance of eczema and acne in the former and fungal and bacterial infections in the latter. In addition there are often regional differences such as the prevalence of scabies in certain areas, particularly in Latin America.[17]

The main fungus isolated was *Trichophyton rubrum* which is commonly found in South America including areas in Colombia with similar climatic conditions.[21,22] However, the morphology of the isolates was granular with scattered microconidia and numerous macroconidia. This appearance is unlike that normally seen in European or U.S. isolates, but similar to those reported from a number of tropical areas such as Fiji,[23] Hong Kong[24] and Nigeria.[25] In all cases the organism was isolated from more inflammatory forms of tinea corporis than is usual for *T. rubrum* infections. The colonies were smooth at first in culture with deep red pigmentation. The presence of a similar organism in lesions of tinea corporis in South America is of interest, as it might suggest the predominance of morphologically distinct strains of this species in similar tinea corporis lesions occurring within a very wide geographic range. The prevalence of pityriasis versicolor has been commonly reported from areas with similar climates.[17] In addition one patient had an infection of the finger nails caused by *Hendersonula toruloidea*,[26] the first time to our knowledge that this infection has been diagnosed in Ecuador.

In our survey, skin infections and infestations such as scabies were the most commonly recognized forms of disease, hand eczema being found in only two individuals. Because of serious contamination with Gram negative bacteria originating from the river only a few of the skin lesions yielded significant bacterial pathogens, in most instances *Staphylococcus aureus*. Potentially pathogenic streptococci were only found in lesions of pyoderma in two cases, although different sampling techniques such as the use of silica gel treated swabs might be necessary to establish the true incidence of streptococcal infection. These findings are in contrast to the pattern of infection found in other tropical areas,[27] and the apparent absence of streptococcal pyoderma may be due to the fact that other bacteria replace the initiating streptococci in later lesions. The prevalence of penicillin resistant *S. aureus* in wounds is of interest. It has been shown that penicillin resistance in both military and civilian populations with staphylococcal pyoderma in Vietnam occurred in between 80–85% of all those tested. Tetracycline resistance was seen in 40–65%.[27] This pattern is thought to reflect the availability of antibiotics and, therefore, of patients' exposure to antibiotics. In the study population in Ecuador antibiotics are less readily available, although both oral penicillin and topical tetracycline ointment are used even in this remote area. Most of the infections were associated with scabies or bites. However, in infants large infected lesions on the scalp were common and their resistance to therapy, usually oral penicillin or tetracycline ointment, well recognised. There was no evidence that there was heavy colonization of onchodermatitis lesions with *S. aureus* as may occur in atopic eczema. This has been confirmed subsequently in a larger population of patients with lichenified onchodermatitis in Eastern Sudan.

At present this population has not received mass treatment with the drug ivermectin, which has been more extensively used in Africa, and it will be important to document the effect of such therapy on the pattern of skin disease in areas endemic for onchocerciasis.

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A controlled trial of house dust mite eradication using natamycin in homes of patients with atopic dermatitis: effect on clinical status and mite populations

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SUMMARY

In a controlled clinical trial, patients with atopic dermatitis carried out house dust mite eradication procedures on their mattresses, using natamycin or a matched placebo spray, with or without vacuum cleaning, for 4 months. For the two groups that used vacuum cleaning, mite numbers fell significantly both by comparison of mean initial and final numbers and by calculating mean rates of reduction from regression analyses ($P < 0.01$ in all cases). There were no differences between the groups in the magnitude of the decreases. For the two groups that did not use vacuum cleaning, mite populations remained virtually unchanged. These results indicate vacuum cleaning not natamycin had the major effect on mite eradication.

Mean symptom scores of patients in all four groups were slightly reduced by the end of the trial, but there was a greater reduction rate ($P < 0.01$) in the combined scores of the groups that did not use vacuum cleaning, demonstrating a lack of correlation between improvement in clinical score and lowered mite numbers. No patients entered remission and the maximum improvement in clinical score was only 47%. Natamycin and vacuum cleaning neither alone nor in combination proved valuable in reducing mite numbers sufficiently to provide clinical benefit.

There is strong circumstantial evidence that house dust mite allergens are an important precipitating factor for atopic dermatitis. This includes mite-specific IgE immunoglobulinaemia in patients with atopic dermatitis[1] and correlation between clinical severity and specific serum IgE levels;[2] clinical improvement after mite avoidance;[3] effective immunotherapy with mite extracts;[4] and development of eczematous reactions following percutaneous challenge with

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mite allergens.[5,6] In view of such compelling evidence it is surprising that the present study is, to our knowledge, the first controlled double-blind clinical trial of mite eradication in homes of patients with atopic dermatitis.

Natamycin is an antifungal antibiotic used for topical mycoses. It works as an acaricide by killing the house dust fungi which digest lipids in human skin scales, the scales being the food of house dust mites. Without fungi the lipids remain at levels toxic to the mites.[7,8] Natamycin is available as an aerosol spray under the brand name 'Tymasil' (Brocades (Great Britain) Ltd. West Byfleet, Surrey, U.K.). It has been used in several clinical trials for control of mites in homes of mite-hypersensitive patients with asthma and/or rhinitis, both with clinically beneficial results[9] and with no benefit.[10]

METHODS

Design of trial, patient eligibility and clinical assessment

Twenty-three patients living in and around Greater Glasgow (Scotland) and attending the Dermatology Clinic at the Western Infirmary were entered into the study. Two left the study during the pre-trial period (week −2) and one left during week +2. Their data were not included in the analyses. All patients had the classical clinical features and chronic relapsing course of atopic dermatitis and their diagnostic features are shown in Table 1. They showed positive immediate skin prick tests to house dust mite extract, confirmed by positive RAST for anti-house dust mite (*Dermatophagoides pteronyssinus*) IgE, as well as elevated total serum IgE levels. Patients were seen at the Dermatology Clinic at −2, 0, +4, +8 and +12 weeks. At each visit clinical severity of eczema was scored on a scale of 0–3 for six features (extent, erythema, pustulation, excoriation, dryness and cracking) at 16 sites on the body. At the initial and final

TABLE 1. Atopic history, sexes and ages of patients

Treatment group	NV	Nv	nV	nv	Total
Number of males:females	3:3	3:1	1:4	4:1	11:9
Mean age (years)	24·8	30·0	31·0	24·6	
Age range	12–46	13–47	18–43	14–40	
Age of onset of AD (years)					
<2	6	3	3	4	16
2–5	—	—	—	1	1
>5	—	1	2	—	3
Personal history of atopy					
Asthma	4	2	4	2	12
Rhinitis	4	2	4	4	14
Urticaria	1	—	2	—	3
Conjunctivitis	1	—	—	—	1
None	—	1	—	1	2
Family history of atopy					
Eczema	—	2	3	2	7
Asthma	3	1	1	2	7
Rhinitis	1	—	1	1	3
None	2	2	1	1	6

visits, 10 ml of venous blood was taken for serological investigation (see below) and they were also skin prick tested using extracts of house dust mite (*D. pteronyssinus*), grass pollen, cat and dog dander and mould (*Aspergillus fumigatus*) (Bencard Ltd., Brentford, U.K.).

Patients were assigned randomly to one of four treatment groups (figure in brackets: number of patients who completed the trial): active natamycin spray and vacuum cleaning (NV; 6); active natamycin spray and no vacuum cleaning (Nv; 4); inactive matched placebo spray and vacuum cleaning (nV; 5); and inactive matched placebo spray and no vacuum cleaning (nv; 5). This scheme allows for the combination of data from any two groups for comparison with that of the remaining two groups.

Patients were entered into the trial between 23 November 1984 and 21 February 1985. The entire trial lasted from 23 November 1984 to 18 June 1985.

Serology

Serum samples were stored at −20°C. All sera from each patient were assayed in one session by RAST for IgG and IgE against house dust mite (*D. pteronyssinus*,) and IgE against mould (*A. fumigatus*) and grass pollen (*Poa pratensis*). Total IgE was assayed by IgE radioimmunoassay. Any non-specific binding of high serum IgE (expected in patients with atopic dermatitis) to the allergen-coated discs was assessed by binding to discs coupled with human serum albumin (HSA) (Lot no. 29805). All assays were carried out using commercial kits (Pharmacia Ltd., Uppsala, Sweden) following the instructions of the manufacturer.

Mite eradication measures

Mite eradication was restricted to the patients' bedrooms. Preliminary measures included the removal of house plants, soft toys, cushions and, wherever possible, upholstered furniture other than beds. The number of pillows was standardized to one per bed occupant. Spraying of the beds with natamycin or placebo was done by the patients and followed the manufacturer's instructions. Briefly, the upper surfaces of the mattresses and both sides of the pillows were sprayed every 2 weeks and aired for half an hour afterwards. One aerosol can containing 625 mg of natamycin was used for spraying a single bed and two cans for a double bed. Actual natamycin dosage was checked in the laboratory by discharging 20 cans and calculating the mean dry weight of the contents.

Sprayings were recorded on a diary card by the patients. They received full written instructions and a demonstration of the procedure. Patients using vacuum cleaning all possessed hose-type vacuum cleaners (carpet beater-type vacuum cleaners are not suitable for mattresses). They cleaned the upper surface of the mattess and both sides of the pillows just before each spraying.

Monitoring of house dust mite population change

An initial visit for dust sampling was made (−2 weeks) with four subsequent visits at weeks 0, +4, +8 and +12, coinciding as near as possible with the patients' attendance at the Dermatology Clinic.

Methods of dust sampling, and mite extraction followed those used previously.[11] Mites were mounted on microslides, using the frozen block technique,[12] in Hoyer's medium and identified using a compound microscope. Mites were also recorded as 'damaged' or 'intact' as an estimate of whether they were alive or dead at the time of sampling.

Statistics

House dust mite counts and clinical scores for weeks 0–12 were converted into ratios of those for week −2. After square root transformation, regression analysis was used to obtain the lines of best fit and the values of the slopes (rate of change) and intercepts were calculated. Having checked that the intercepts were on or around 1, the rates of change were treated with two-way unbalanced analysis of variance. The effects of the two treatment types were analysed using *F*-tests.

Data on skin prick tests and serology were analysed using paired *t*-tests.

RESULTS

House dust mite eradication

Dermatophagoides pteronyssinus and *Euroglyphus maynei* accounted for 97% of the total mite specimens recovered from the dust samples.

The geometric mean numbers of total house dust mites in mattresses fell by 1·9 times by week +12 in group NV and by 3·1 times in group nV (Table 2) but by only 1·08 and 1·02 times in the groups that used natamycin only and placebo spray only. Numbers of intact mites increased in both these groups by week +12.

The geometric mean rates of change of total mite numbers in mattresses, as calculated from lines of best fit, were −25·8% in 12 weeks for group NV, ($P < 0·01$), −50·3% for group nV ($P < 0·01$), +15·1% for group Nv and −24·9% for group nv (*P* not significant in both cases) (Fig. 1). There was no significant difference between the rate of reduction in group NV and group nV. Analysis of the separate and combined effects of vacuum cleaning and natamycin demonstrated that vacuum cleaning and not natamycin had the major effect in reducing house dust mite numbers ($P < 0·01$).

TABLE 2. Geometric means of total house dust mites (all spp., damaged plus intact)/0·25 m^2/min from mattresses of each patient

	Week of sampling				
	−2	0	+4	+8	+12
Treatment group					
Natamycin, vacuuming (NV)	63·6	45·6	76·7	54·8	34·2
Natamycin, no vacuuming (Nv)	385·3	270·2	419·8	302·8	357·4
Placebo, vacuuming (nV)	47·5	42·7	33·1	36·9	15·0
Placebo, no vacuuming (nv)	144·1	225·6	211·2	154·4	141·1

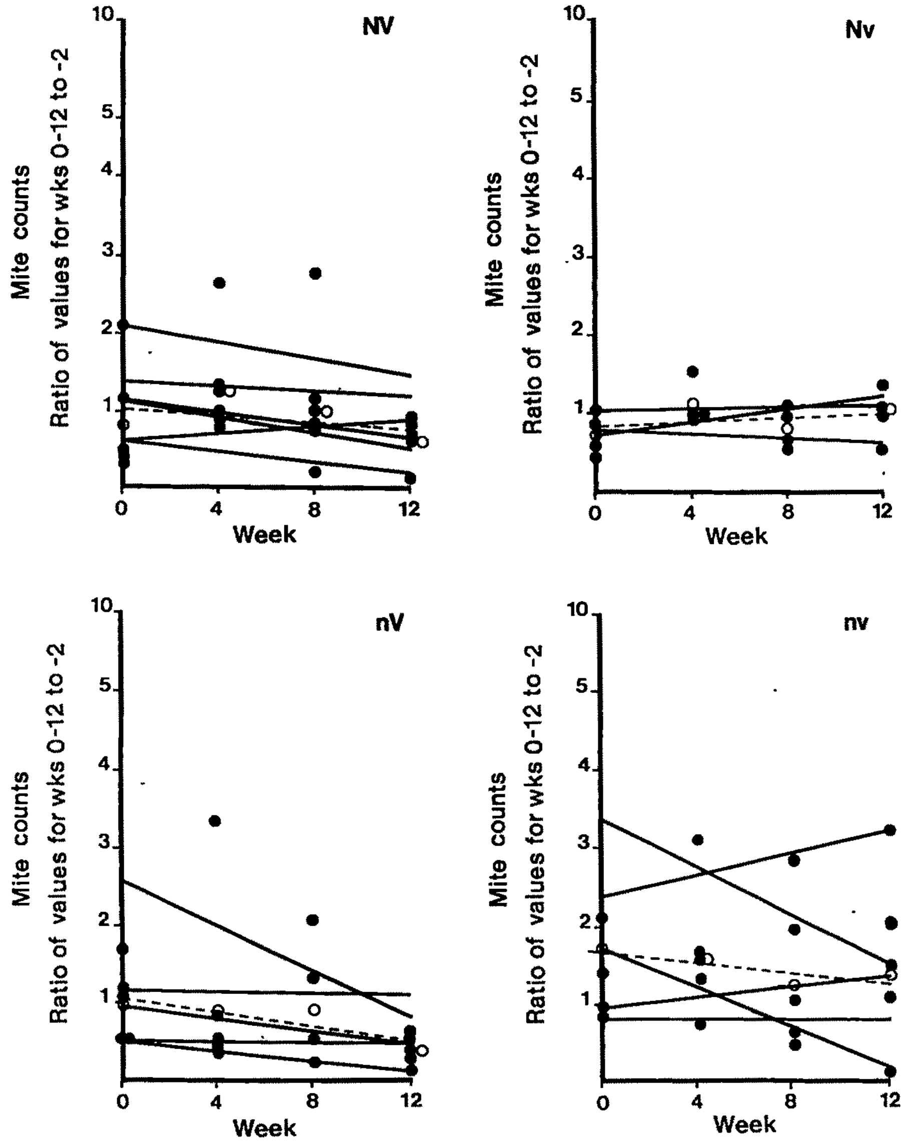

FIGURE 1. Rates of reduction of total house dust mite numbers represented by lines of best fit derived from regression analysis of ratios of scores for weeks 0–12 to scores for week −2. ●, individual values; ○, geometric mean of individual values. ----, line of best fit for geometric means.

Symptom scores

Arithmetic mean symptom scores in all four treatment groups were slightly reduced by week +12 compared with week −2 scores (Table 3). The mean rate of change of symptom scores, as calculated from lines of best fit (Fig. 2), showed only group Nv had a greater percentage improvement rate (24·0% in 12 weeks) than the control group, nv (20·4%). Improvement rates for groups NV and nV were 8·4% and 0·7% respectively.

TABLE 3. Arithmetic means of symptom scores ± 95% confidence intervals

	Week of clinical examination				
	−2	0	+4	+8	+12
Treatment group					
Natamycin, vacuuming (NV)	55·2 ± 12·5	46·2 ± 25·8	47·3 ± 43·9	42·3 ± 41·2	38·6 ± 37·1
Natamycin, no vacuuming (Nv)	55·3 ± 49·3	52·0 ± 38·5	53·8 ± 53·4	45·0 ± 41·7	43·8 ± 41·3
Placebo, vacuuming (nV)	57·4 ± 20·6	47·8 ± 33·4	62·0 ± 42·0	58·8 ± 52·5	51·0 ± 44·5
Placebo, no vacuuming (nv)	45·2 ± 18·6	46·6 ± 35·6	43·0 ± 33·9	40·2 ± 27·2	35·8 ± 26·4

When the effect of vacuum cleaning and natamycin on symptom scores was analysed, a significantly greater rate of reduction ($P < 0{\cdot}01$) was found in groups that did not use vacuum cleaning.

No patients went into remission. Four (67%) in group NV had positive improvement rates at week 12; four (100%) in group Nv; two (40%) in group nV and five (100%) in group nv. The greatest rate of increase in clinical score, as calculated from lines of best fit, was only 16% for a patient in group nV whilst the greatest decrease was 47% for a patient in group nv.

Skin prick tests

Skin prick testing was completed for 16 of the patients (Table 4). All four groups showed no significant change in geometric mean area of weals raised to any of the test extracts by week + 12 even though three patients in group NV had > 2-fold reductions in weal area to *D. pteronyssinus* by then. All patients were skin prick test positive to house dust mite, 15 (94%) to grass pollen and to cat dander, 12 (75%) to mould and 11 (69%) to dog dander. All but one patient showed a positive reaction to at least four preparations.

Serology

Levels of total serum IgE, and anti-*D. pteronyssinus* IgG and IgE were elevated in all 20 patients (Table 5). Geometric mean levels declined in all four groups by week 12. There were statistically significant reductions for anti-*D. pteronyssinus* IgE in group NV ($P < 0{\cdot}05$), and for anti-*D. pteronyssinus* IgG and total IgE in group nV ($P < 0{\cdot}01$ and $< 0{\cdot}05$ respectively). The only other statistically significant changes were reductions in mean anti-*Poa pratensis* IgE in groups nV ($P < 0{\cdot}02$) and nv ($P < 0{\cdot}05$).

Natamycin dosages

Arithmetic mean surface areas of the upper side of a single bed plus one pillow and a double bed plus two pillows were 2·24 and 4·21 m^2 respectively. Arithmetic mean delivered dosage (dry weight) of natamycin was 574 mg per can (range 369–688 mg). Representative doses were thus 256 mg/m^2 for single beds and 273 mg/m^2 for double beds.

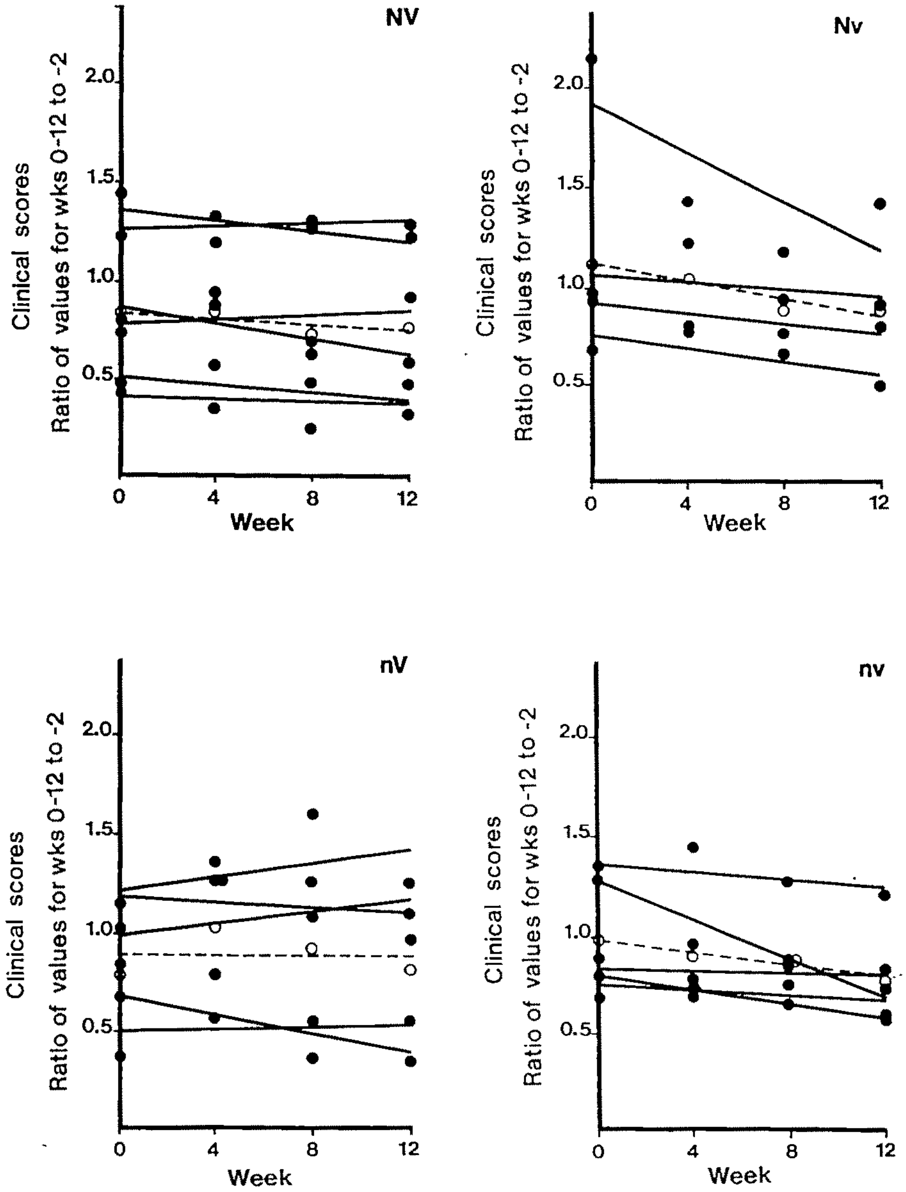

FIGURE 2. Rates of reduction of symptom scores represented by lines of best fit derived from regression analysis of ratios of scores for weeks 0–12 to scores for week −2. Symbols as in Figure 1.

DISCUSSION

In this trial the major effect on mite eradication was due to vacuum cleaning rather than to natamycin. There was no clear synergistic effect from combining the two treatments and natamycin had only a slight acaricidal effect. Recently Saint Georges-Gridelet reported that double the recommended dosage of 300 mg/m^2 was required for persistent fungicidal activity and efficient mite control[8] and suggested that inefficacy was caused by irregular distribution of

TABLE 4. Skin prick test results at weeks −2 and +12. $\bar{x}$ = geometric means of wheal area (mm²) minus control, n = number of patients positive

	Allergen extract									
	HDM		Cat		Dog		Grass		Mould	
	−2	+12	−2	+12	−2	+12	−2	+12	−2	+12
Treatment group										
Natamycin, vacuuming (NV)										
n	6	6	4	6	3	3	6	6	2	3
$\bar{x}$	30·4	18·7	5·1	9·8	1·9	2·1	18·7	12·3	1·9	2·6
Natamycin, no vacuuming (Nv)										
n	3	3	2	3	1	2	3	3	1	2
$\bar{x}$	32·4	35·4	4·3	5·5	1·6	2·0	6·3	13·6	1·7	2·7
Placebo, vacuuming (nV)										
n	3	3	2	3	2	2	3	3	1	3
$\bar{x}$	35·2	28·4	3·0	7·3	2·2	6·5	9·8	18·2	1·4	4·4
Placebo, no vacuuming (nv)										
n	4	4	3	0	3	4	3	3	2	4
$\bar{x}$	29·2	28·1	6·3	10·2	3·3	8·3	7·0	6·1	1·9	2·9

TABLE 5. Serological results at weeks −2 and +12. Geometric means. Units: IgG = arbitrary units, IgE = International RAST units, Total IgE = International RAST units/ml

	Assay											
	HDM IgG		HDM IgE		Grass IgE		Mould IgE		Total IgE		HSA	
	−2	+12	−2	+12	−2	+12	−2	+12	−2	+12	−2	+12
Treatment group												
Natamycin, vacuuming (NV)	61·0	54·7	88·2	74·0	44·1	25·0	1·7	1·6	8939	5930	0·2	0·2
Natamycin, no vacuuming (Nv)	27·3	25·0	94·9	71·7	56·9	30·3	1·6	1·9	9980	4583	0·3	0·1
Placebo, vacuuming (nV)	48·5	42·7	69·1	51·7	62·9	35·2	1·1	0·9	8998	6784	0·2	0·1
Placebo, no vacuuming (nv)	74·7	82·5	63·6	48·4	6·2	4·5	0·8	0·6	3284	2061	0·1	0·1

natamycin after spraying. We observed that patients often overestimated the time it took the aerosol can to empty and this did indeed lead to patchy spraying. We also found that many of the cans had not delivered their full contents before the propellant was exhausted.

The lack of correlation between improved symptom scores and reductions in mite numbers in mattresses and pillows has three major implications. Firstly, the decrease in clinical score of patients in the control group indicates a placebo effect due to the substantial intervention involved in the trial. The encouraging reductions in symptom scores achieved by August[13] and Roberts[14] in uncontrolled trials of mite avoidance may likewise be due in part to intervention as well as reduced allergen exposure. Secondly, the greatest improvements in mean symptom scores were in the groups which did not use vacuum cleaning. This may have been because vacuum cleaning disperses fine dust particles in the outlet aircurrent.[15] Allergen-containing dust is sucked up from sub-surface locations, e.g. the insides of mattresses, dispersed into the air and then deposited onto surfaces where it may be picked up on the patients' skin. Thirdly, we have attempted to control only one source of allergens: atopic dermatitis is a multi-factorial disease and many other stimuli such as infection, stress and a variety of other allergens may all cause a flare of the disease and obviate the beneficial effects of lowered numbers of house dust mites.

Were the trial to be repeated, a longer study period with more participants, combined with more efficient acaricidal methods, would be preferable. The success of any mite eradication method is dependent on patients following the protocol correctly. Three patients admitted to single omissions of sprayings and cleanings. To prevent this problem we would suggest that all eradication procedures be carried out by a member of the clinical trial team.

It has been proposed that significant improvement in symptoms of house dust mite-mediated asthma require at least a 10-fold decrease in allergen exposure and an arbitrary hygiene standard of below 100 mites per g of dust, or 2 μg of allergen *Der p*I, per g has been set.[16] We have found that natamycin cannot achieve such reductions when used at the recommended dosage. For patients with atopic dermatitis reductions in mite exposure even greater than 10-fold may be necessary, as marked improvement in symptoms has so far only been achieved by removing patients from their normal environment, usually to hospital where house dust mite numbers are very low.[3,13,17] In the future more efficient acaricidal methods may prove to be of benefit to patients with atopic dermatitis.

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Psychological disabilities amongst patients with port wine stains

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SUMMARY

In a questionnaire study to assess psychological disabilities in 110 patients with port wine stains, 71 completed questionnaires were analysed. The questionnaire revealed a high level of psychological morbidity resulting from a feeling of stigmatization amongst these patients that was not apparent in casual social intercourse. In contrast, standard psychological screening tests for psychiatric illness, depression or anxiety revealed that these patients had similar or less evidence of morbidity than controls or other patient groups. It would appear that patients with port wine stains suffer a considerable degree of psychological morbidity which they suppress. This may be related to a need to hide their port wine stain.

Port wine stains are benign, vascular naevi, consisting of ectatic dermal blood-vessels. The naevus appears pink at birth and persists, often darkening with advancing age to a purple colour and nodules may develop. The affected part may hypertrophy. As most port wine stains occur on the face, an area of major importance in self-image, they present a considerable cosmetic burden to the patient, and are noticeable to other people within the patient's environment.

Body images of oneself and others are of fundamental importance in the development of personality and interpersonal relationships.[1] In our society attractiveness is often rated more highly than other aspects of the personality[2,3] and people who are attractive tend to reap more of life's benefits than those less well endowed.[4] In addition, society is prejudiced against deformity, even if in an unconscious manner.[5,6]

Birthmarks such as port wine stains were taken in mediaeval times as evidence of the bearer's service to the devil.[7] Whilst most patients with port wine stains and the people they meet will be unaware of this historical background, these facts remain in the roots of modern consciousness and even in modern society, persons with an external blemish may be construed as showing evidence of a defective moral character.

Thus, there would appear to be ample reason for a patient with a port wine stain to show evidence of significant psychological morbidity in terms of stigmatization, poor self image and being an underprivileged member of society. However, in our experience in treating a large

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number of port wine stains in the laser clinic at the Leeds General Infirmary, the vast majority seem well adjusted and psychologically normal people. A small number of patients, however, show personality changes when removing cosmetic camouflage, becoming withdrawn and avoiding eye contact and others show ambivalence to treatment, either rapidly switching to and from a decision to undergo therapy or expressing no interest in therapeutic outcome.

It would appear, therefore, that either, despite all the significant problems of a port wine stain, most patients suffer no psychological difficulties, or these difficulties are suppressed and not apparent in casual social intercourse. The aims of this study were to investigate whether there was indeed evidence of psychological morbidity amongst patients with port wine stains by the use of a questionnaire designed to elicit feelings of stigmatization, poor self regard and difficulties in interpersonal relationships. It was hoped to determine whether this morbidity was related to the physical attributes of the port wine stain as determined by the patient, and to establish a baseline psychological measurement early in laser therapy for comparison with repeated measurement on completion of laser treatment.

METHODS

To assess patients' attitudes to their birthmark, a questionnaire was devised in conjunction with the Department of Psychological Medicine. The anonymous questionnaire contained 16 general questions which allowed identification of the respondent, and an assessment by the patient of the severity of the birthmark, e.g. the extent of skin involved, was the birthmark flat or bumpy? Was it getting darker or lighter? There followed 26 direct questions requiring a yes/no answer, e.g. 'I feel like an outcast because of my birthmark', 'I am resentful of people with normal skin'. A yes/no format avoided allowing answers such as 'sometimes' or 'rarely' and required a positive commitment to respond. Patients were requested to answer all questions.

Initially, to test the reproducibility of individual responses, 20 patients attending the laser clinic were given two questionnaires each and requested to complete them both, separated by an interval of 2–4 weeks. Using the Spearman rank test, on each question, there was a very high correlation for each of the 26 paired responses (lowest correlation $r=0{\cdot}88$, $P\ll 0{\cdot}001$).

The questionnaire was then administered to 90 patients over the age of 15 years with port wine stains attending the clinic. The questionnaire was given by hand to 30 patients, and sent by post with an explanatory letter to 60 patients selected on an alphabetical basis from the clinic notes. Many of these latter patients had been discharged from the clinic or had failed to attend and had not been seen for many months.

In addition, two standard psychiatric screening tests, the general health questionnaire (GHQ) and the hospital anxiety and depression (HAD) scale were given to the first 30 and 25 patients respectively. Twenty-nine patients completed the questionnaire delivered by hand, and 22 of 60 returned the posted questionnaire. These 51 questionnaires, plus one of each of the first 20 pairs, were analysed—a total of 71 out of 110 patients contacted (response rate 64·5%).

RESULTS

Details of the respondants and the assessment of their port wine stain are shown in Tables 1 and 2. The age, sex and site of the port wine stain was not significantly different in those patients not replying. By identifying each respondant, it was possible to categorize each patient's current status in their laser treatment and this is shown in Table 3. Four patients with good results had completed laser treatment.

TABLE 1.

No = 71 (Females 46, Males 25)

Age	
15–25	27 (13 F, 14 M)
26–35	21 (16 F, 5 M)
>35	23 (17 F, 6 M)

Employed: 39; Housewife: 12; Unemployed: 10; Student: 5

TABLE 2. Assessments of port wine stain

Site
Face 65; Other 6; Unilateral 64; Bilateral 7
Colour
Pink 11; Red 38; Purple 22
Changes observed
None 25; Darker 19; Lighter 12; Grown 11; Shrunk 4

TABLE 3. Patient status related to laser treatment

No treatment	14
Test treatment, no result	9
Good result	31
Poor result	12
Other	5

The use of cosmetic camouflage makeup is shown in Table 4. It can be seen that few men use camouflage makeup. In patients who do use it, the majority find it at least partly successful. Twenty-five patients would not go out without make-up and four women would not even show their birthmark to their husbands.

Thirty-three patients had received other treatment to their port wine stain, but only four had considered it successful.

Psychiatric screening tests

The General Health Questionnaire is a screening questionnaire designed for detecting psychiatric illness within a population.[8] The 30-item GHQ was used in this study and three of 30 patients questioned gave an abnormal response (score >4). The Hospital Anxiety and Depression scale is designed to detect emotional disorders in hospital patients.[9] Scores of 7 or less were classified as non-cases, 11 or more as definite cases, and 8–10 as doubtful cases.[10] Four patients scored 8–10 and one patient scored over 10.

In contrast to the screening tests, the questionnaire provided a high percentage of positive responses. Out of a total of 26 questions, the median number of positive responses per patient was 15, with a range of 1–23. The three patients with an abnormal GHQ gave 17, 19 and 23

TABLE 4. Use of cosmetic camouflage

Yes 39 (35 F, 4 M)
No 32 (11 F, 21 M)
Successful Yes 22; No 9; Partly 8
Do you go out without make-up? Yes 44; No 25 (2 Males)
Does your spouse see you without make-up? No 4 (All Females)

positive responses respectively and the five with abnormal HAD scores gave 9, 16, 20, 23 and 23 positive responses respectively.

The two highest response rates were for questions related to treatment; 92% of patients said they would feel better about themselves if their birthmark was improved by treatment and 83% said they would feel more comfortable with members of the opposite sex if their birthmark was improved by treatment.

Questions with a high positive response rate are shown in Table 5. Over 50% of patients also felt embarrassed, anxious or depressed because of their birthmark. Forty-one per cent felt envious of people with normal skin and the same number felt people avoided looking at them. Twenty-three per cent said they avoided sexual activity because of their birthmark.

Least positive responses were to questions relating to work difficulties (15%), having relationships with people of the same sex affected by their birthmark (18%) and people not wanting to be their friend because of their birthmark (20%).

Fisher's exact test was performed to assess any correlations within responders. Only in females and the older age group of patients (Table 6) were there significant correlations with responses.

Patients were invited to add any comments or suggestions on how the questionnaire might be improved. Of those who contributed, most expressed thanks for being allowing to vent long-suppressed feelings. Other observations were illuminating illustrations of the problems these patients are facing, e.g. 'Since I was a young teenager I have never even answered the door without makeup', 'I attempted suicide after several operations on my birthmark went wrong at the age of 21', 'I personally have a dread of looking into mirrors, as a mere glance can change my whole mood from good to bad. I try to use mirrors at home in semi-lit rooms', 'I don't even like talking to my mum about it because I feel guilty for not being normal'.

TABLE 5. Questions with a high positive response rate

Question	+ve response
I feel people stare at me because of my birthmark	75
I have been hurt by what other people say to me because of my birthmark	73
I feel the need to hide my birthmark	72
If my birthmark is improved by treatment, I will feel more comfortable with people of the same sex	71
My birthmark has affected my self-confidence	71
Having a birthmark makes me feel different from other people	67
If my child were to have a birthmark, I would feel guilty	67
I feel physically unattractive and sexually undesirable because of my birthmark	54

TABLE 6. Correlations amongst respondants

Females and:	Cosmetic camouflage	(*)
	Go out with makeup	(*)
	Need to hide birthmark	(†)
	Feel different from other people	(‡)
	Age	N.S.
Older age and:	Feel different from other people	(†)
	Embarrassed	(†)
	Social situations avoided	(†)

*$P < 0.001$ †$P < 0.01$ ‡$P < 0.05$.
N.S. = not significant.

DISCUSSION

A number of studies have been performed to assess psychological and psychiatric disturbances in patients with a variety of skin disease.[11-16] Patients with acne have an impaired self image,[12] and evidence of increased psychological distress which improved with therapy.[13] Patients with severe psoriasis are less socially self-confident than control groups of patients with other skin conditions.[14] Patients with vitiligo exhibited lower self-esteem than normal controls,[15] and patients with melanoma were superior in well-being when compared with other dermatology patients.[16]

However, previous investigations concerning psychological effects of laser therapy in patients with port wine stains using standard tests have revealed that these patients were no different from normal controls,[17,18] although they were found to be perfectionistic and had unrealistic expectations of the outcome of therapy.[19] These patients felt they were at considerable personal, professional and social disadvantage due to their naevus.

The results from this questionnaire study of 71 patients with port wine stains indicate that these patients suffer a significant degree of psychological morbidity. This morbidity was not detected in two standard psychiatric screening tests. There was a 10% abnormal response to the GHQ, which compares with 11% abnormal responses in a general population to a slightly different questionnaire,[8] 30% in women attending a general practitioner,[20] 30% attending a large dermatology out-patient clinic[11] and 45% of medical in-patients.[21]

Using the HAD questionnaire, 20% of patients with port wine stains registered an abnormal score, which compares with 30–40% of hospital outpatients[10] and patients with chest pain referred for exercise testing.[22]

It would appear that patients with port wine stains suffer psychological distress which is not apparent in their social interactions and when applying indirect psychological testing. Patients with port wine stains undoubtedly experience feelings of stigmatization, difficulties in interpersonal relationships, particularly with members of the opposite sex, and feelings of guilt, embarrassment, anxiety and depression. A high percentage of patients said they had been hurt by other people and were stared at. This may be a real occurrence or reflect heightened sensitivity amongst these patients.

It is disturbing that older respondants feel different from other people more often, which is a central feature of stigmatisation; they were more often embarrassed because of their birthmark and avoided social situations because of it. These results suggest that with increasing age, there is little acceptance of the problems associated with a port wine stain and patients may in fact show a failure to adapt to these difficulties.

Nearly three-quarters of the respondents felt the need to hide their birthmark, and this is reflected in the use of cosmetic camouflage in females. Males have considerable difficulties in coming to terms with using make-up. This need to hide their port wine stain may partly explain the mechanism by which patients suppress their psychological difficulties. In a further attempt to conceal their birthmark, these patients normalize their behaviour as if there was no abnormality present. This was confirmed by those patients who added comments to their questionnaire.

The vast majority of patients felt that (laser) treatment of their port wine stain would result in an improvement in the quality of their lives, a similar finding to previous studies,[17,18] and it will be of interest to compare questionnaire responses when patients eventually complete treatment.

In conclusion, this study demonstrates that patients with port wine stains suffer considerable psychological disabilities which they suppress. These difficulties do not improve with advancing age and may cause further problems for patients in adapting to their social environment. Patients exhibit considerable optimism towards treatment of their birthmark, although there is no evidence to date that an improvement in their psychological well-being will ensue. However, clinicians dealing with these patients should be aware of this psychological morbidity and, where possible, an assessment of suitability for treatment should be considered.

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Histology of port wine stains after copper vapour laser treatment

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SUMMARY

We report histological changes in four patients with port wine stains treated with 578 nm yellow light from a high power copper vapour laser. Histology showed that selective damage occurred to the ectatic blood-vessels in the dermis, without haemorrhage and damage to non-vascular structures, and without scarring. The initial damage to the overlying epidermis was not permanent, and the damaged ectatic vessels returned to normal size or were completely necrosed and replaced by collagen.

Histological studies of the results of laser treatment have shown non-specific necrosis of all cutaneous tissue with use of the carbon dioxide laser,[1] or more specific damage to vascular tissue but accompanied often by an unacceptable level of scarring with the use of the argon ion laser.[1,2] More specific vascular damage has been shown to occur with short pulses of the 577 nm tunable dye laser.[1–4] We report the histological changes after the treatment of four patients with port wine stains with exposure to yellow 578 nm light from a copper vapour laser (Quentron™ QM90-51C).

The low ratio of melanin to haemoglobin absorption in the 560–580 nm range suggests that specific heating of ectatic blood-vessels will occur with light in these wavelengths.[5,6] Histological studies[1–4] support the use of yellow light over the wavelength produced by the argon ion laser (principally 488 nm and 514·5 nm), where there is considerably more melanin absorption.[5] Furthermore, the numerical models of van Gemert and co-workers[7–9] and our model[10] suggest that for the ideal damage to ectatic blood-vessels to be produced, the light must be delivered over a specific time interval and this is 1–10 ms.[8,10,11] Some tunable dye lasers delivering pulses with duration approaching this time interval have given good results,[1,2] but the exposure times using the carbon dioxide and argon ion lasers are 100 ms and often longer.

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METHODS

Four Caucasian patients with port wine stains on the face, trunk and extremities were treated with 578 nm light from the copper vapour laser. This is a pulsed laser with a pulse length of 50 ns. However, unlike the pulsed dye lasers, the light is delivered by a large number of pulses in rapid succession (450–1500). The repetition rate is approximately 15 kHz, i.e. one pulse every 67 μs. The pulse train appears to be a very short pulse followed by a comparatively long period, about 1300 times the length of the pulse. Furthermore, each pulse delivers about 0·2 mJ of energy from the yellow light to the skin. This is much smaller than the 100 mJ or more from the single pulse use of the pulse dye laser.[1,4] The energy distribution is also different from the continuous wave laser where the distribution is independent of time.[1,2,12]

The light was focused onto a 1 mm diameter quartz optic fibre which was scanned by hand approximately 2 mm above the skin surface, thus producing a 1·3 mm diameter spot. All the patients were anaesthetized with a general anaesthetic before treatment, because of the large areas to be treated (average 392 cm^2). Light doses of 11–25 J/cm^2 were applied by 1·6–3·6 W of yellow light being scanned across the lesion at a rate of 6–9 s/cm^2. The power used is much higher than that of a continuous wave 577 nm dye laser.[12] The exact dose depended on when the skin first appeared to blanch, this minimal blanching being the endpoint.

Skin biopsies were taken prior to treatment and 24 h after treatment. Further biopsies were taken from the treated areas at 3 or 6 months. The biopsies were processed for light and electron microscopy (Table 1).

RESULTS

Light microscopy

Histology of the port wine stains in all the patients was that of the deeply located type,[13] with ectatic vessels at the junction of papillary and reticular dermis and throughout the reticular dermis. These vessels measured up to 150 μm in diameter.

The pathology of the skin biopsies 24 h following treatment showed an acute inflammatory infiltrate in the dermis, with a subepidermal blister. Parts of the papillary and reticular dermis

TABLE 1. The operating parameters and histologies performed for each biopsy

						24 h post		3–6 months post	
Patient	Age at biopsy (years)	Distribution of port wine stain	Area of port wine stain field cm^2	Type of microscopy	Pretreatment biopsy?	Scan rate s/cm^2	Dose J/cm^2	Scan rate s/cm^2	Dose J/cm^2
A	9	right-face, neck, shoulder, arm	371	light	yes	9	20	9	20
B	18	left-face, neck, lower leg	430	electron	yes	7	11	9	19
C	47	left-face, neck, cheek, forehead, leg, right-face, neck	115	electron	yes	6	17	No biopsy taken	
D	45	left-forearm, arm, neck, shoulder	570	light	yes	7	25	No biopsy taken	

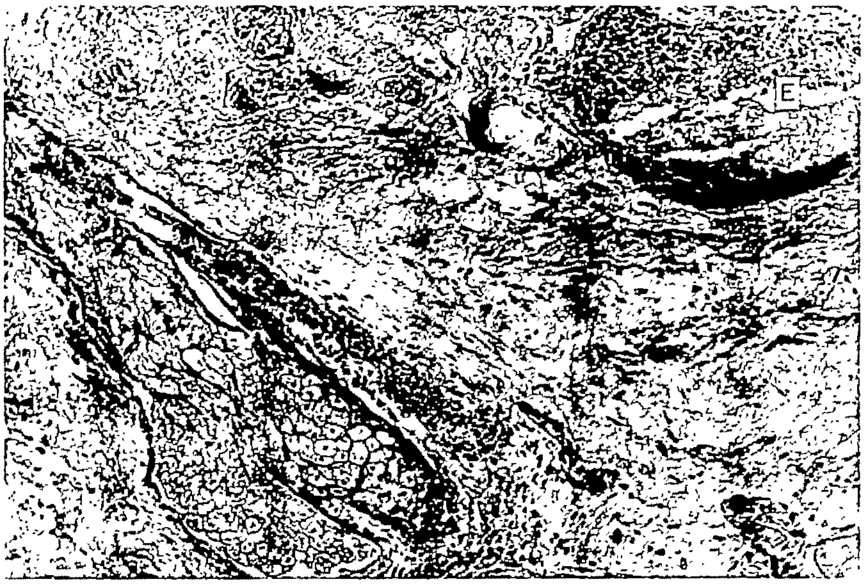

FIGURE 1. Normal appearing hair follicle and sebaceous gland of patient D are adjacent to a coagulated ectatic vessel (E). (Original × 6·5).

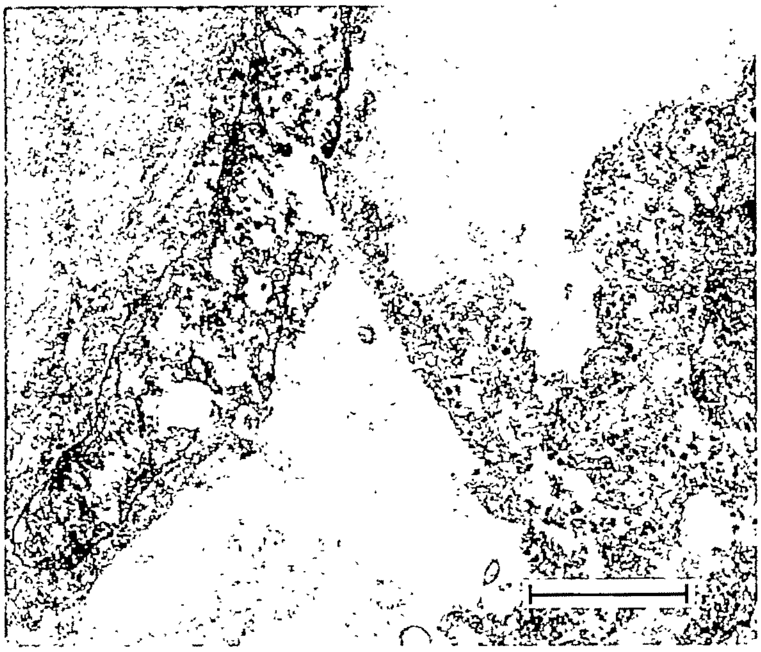

FIGURE 2. Disintegration of the nucleus of an endothelial cell of patient C at 24 h (magnification × 9912; bar indicates 2 μm).

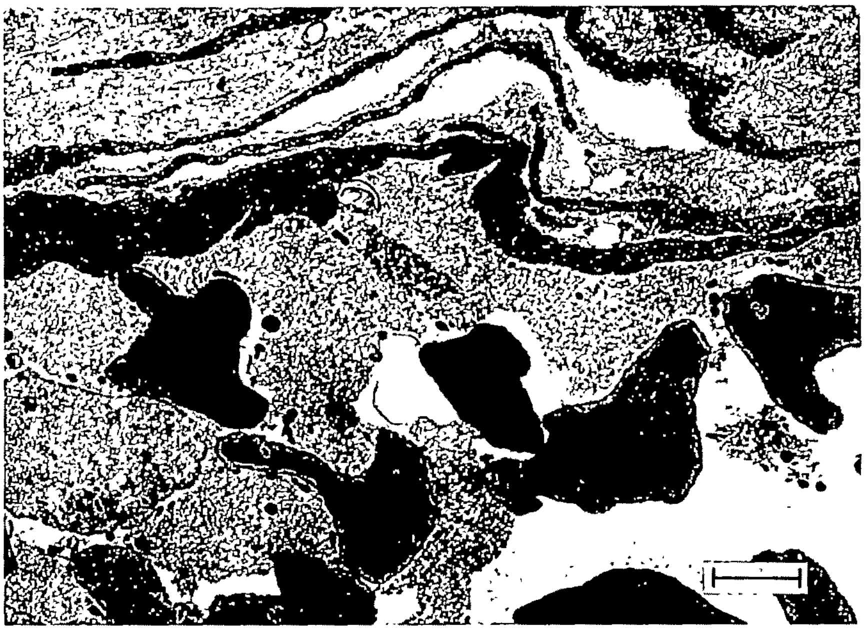

FIGURE 3. Thrombus of red blood cells within an ectatic vessel of patient B at 24 h. Dark spots within red blood cells indicate precipitates of iron (magnification × 5866; bar indicates 2 μm).

appeared to be degenerate and many of the ectatic vessels throughout the papillary and reticular dermis had necrotic endothelial cells, though they still contained red blood cells indicating blood flow. Several of these vessels were more damaged on their top outward facing side, rather than below. Some of these vessels contained a coagulum consisting of protein or neutrophils or both. There was no evidence of rupture of vessels or haemorrhage. Hair follicles, sweat and sebaceous glands appeared normal immediately adjacent to the damaged blood vessels (Fig. 1).

After 3 to 6 months, the epidermis was normal and there appeared to be a normal distribution of melanocytes. The papillary and reticular dermis appeared normal and there was clearance of ectatic blood-vessels. There was a slight increase in the number of normal sized capillaries. No haemosiderin was demonstrated in the dermis. The sweat glands and hair follicles appeared normal.

Electron microscopy

At 24 h after treatment, the endothelial cells showed marked damage, with disruption of the nuclei and considerable damage to the subcellular organelles (Fig. 2). The vessel walls showed degeneration with disruption of basal lamina, and there was destruction of pericytes and extension of the damage to the surrounding collagen fibrils. In some of the vessels there was thrombus with agglutinated red blood cells and there were a few platelets to be found on the walls of some of the larger vessels. A few red blood cells showed evidence of rupture, while others appeared to contain precipitates of iron (Fig. 3). Small vessels were present without the degenerative changes as seen in the larger ones. The epidermis showed some necrosis.

At 3 to 6 months the epidermis appeared to be normal (Fig. 4). There was some increase in the

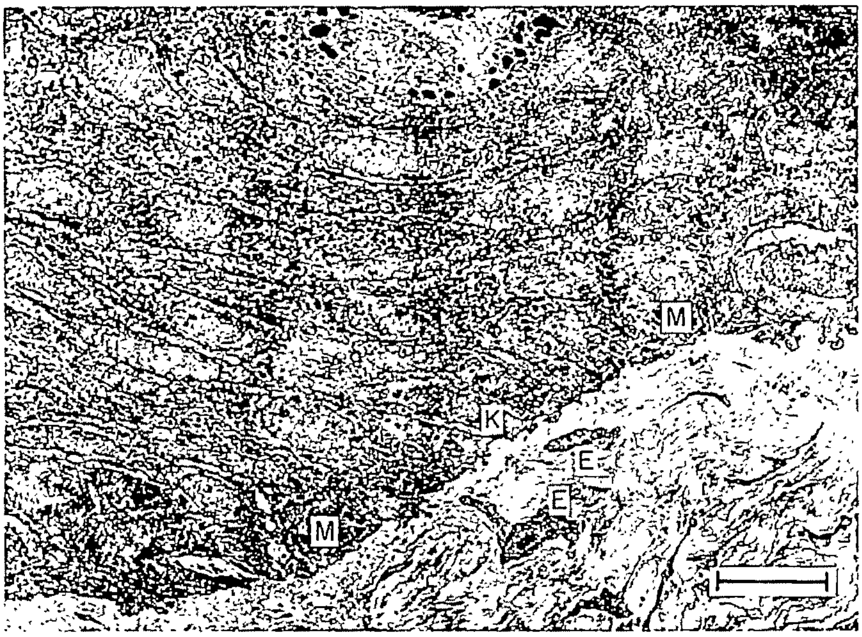

FIGURE 4. Normal epidermis with normal 1 to 7 distribution of melanocytes (M) to keratinocytes (K) of patient B at 6 months. Note the intact endothelial cells (E) apart from any blood-vessel (magnification × 1463; bar indicates 10 μm).

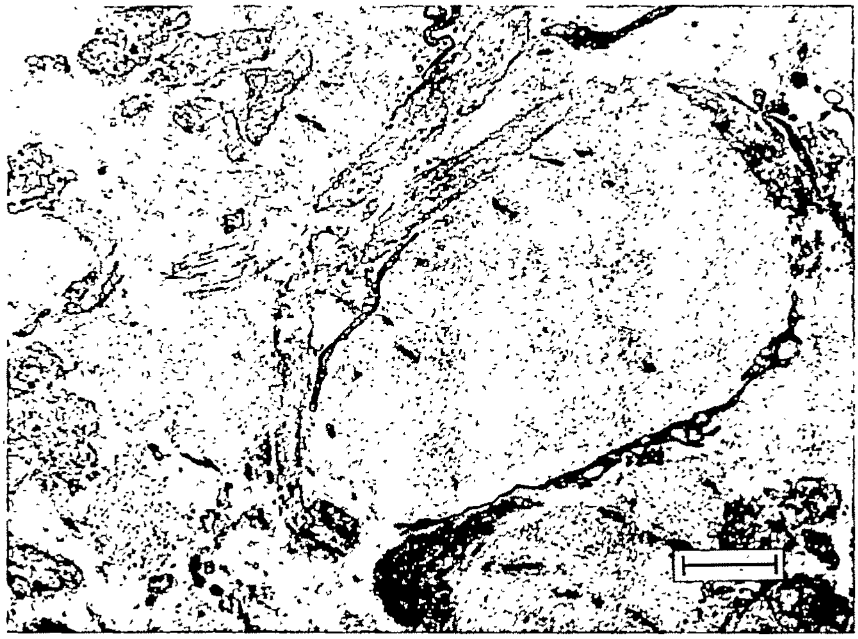

FIGURE 5. A large (approximately 100 μm diameter) blood-vessel that has been destroyed by laser treatment. This shows the vessel of patient B at 3 months completely filled with collagen (magnification × 595; bar indicates 20 μm).

fibrous connective tissue, though the collagen fibrils appeared normal. Some intact endothelial cells and remnants of basal lamina were seen, suggesting that following the occlusion of the ectatic vessels they had been replaced by fibrous connective tissue (Fig. 5). The vessels in the deeper dermis showed marked subendothelial fibrosis, some showing complete occlusion. Smaller vessels in the papillary dermis appeared normal. The endothelial cells of these vessels were often plump and contained normal organelles and many micropinocytotic vesicles.

DISCUSSION

When considering the interaction of light with tissue we need to consider the relationship between several timescales. First, the time over which the light is incident upon a blood-vessel, t_i; second, the pulse length if any of the laser delivering the light, t_p; third, the time required for a blood-vessel raised to a temperature above its immediate surroundings, to fall to a temperature midway between that temperature and the initial temperature of its surroundings: the blood-vessels' thermal relaxation time, t_v.

The argon ion laser is a continuous beam laser and is typically used with $t_i(\mathrm{Ar}^+)$ of hundreds of milliseconds. The histology indicates marked epidermal damage[1,2] and diffuse dermal collagen coagulative necrosis to a depth in the dermis depending on the incident energy density.[2] Destruction of hair follicles, sweat glands and sebaceous glands has been shown to occur. Epidermal and dermal damage is often not repaired and as a result hypertrophic scars have often formed.[1,2] The pumped dye laser is a pulsed laser with pulse lengths in the range $300\ \mathrm{ns} \leqslant t_p(577) \leqslant 360\ \mu\mathrm{s}$. The common histological results are selective damage to blood vessels,[1–4] haemorrhage,[2–4] agglutination of red blood cells,[1–4] and some red blood cell rupture.[2,4]

Thermal relaxation times of blood-vessels varies according to size, and is independent of the incident light. For example blood-vessels with a 20 μm diameter have $t_v = 0{\cdot}15$ ms while those of 60 μm diameter have $t_v = 1{\cdot}3$ ms.[5] The copper vapour laser is a pulsed laser with very short pulses (50 ns) repeated very rapidly (15,000 per s—15 kHz). The high repetition rate means that even for the smallest ectatic vessels many pulses are absorbed by each vessel during the relaxation time t_v, and we are therefore able to consider the laser as operating in an essentially continuous mode. The illumination time $t_i(\mathrm{Cu})$ is determined by the rate of scan of the light across the treated area; we operated with $30\ \mathrm{ms} \leqslant t_i \leqslant 100$ ms.

As with the argon laser, initial epidermal damage was seen to occur, consisting primarily of superficial blistering. However, unlike with the argon laser,[1,2] histology at 3 months indicated that this damage was not permanent. By the use of shorter illumination time scales, and because there is 30% less absorption by the melanin, considerably less heating was caused within the epidermis. Furthermore, the yellow light is four times more strongly absorbed by the haemoglobin than green light, so the outer crescent of ectatic vessels was heated most and hence the heating of the capillary wall was much more specific. Those vessels showing damage to their top side were a direct consequence of the strong absorption of yellow light.

The dye laser histologies[1–4] produced selective damage of blood-vessels similar to the present results with the copper laser. With the copper vapour laser the immediate observed response of the skin is blanching and it appears that damage was the result of heat conduction from the haemoglobin, causing thermal necrosis of endothelial cells and thermal damage to localized perivascular fibrous connective tissue. There was no visual or histological evidence of haemorrhage and only very little thrombosis or blood cell rupture. Rupture of blood cells and haemorrhaging is indicative of the very short (submillisecond) time-scale heating that occurs when the full dose of energy is applied essentially instantaneously, before the red blood cells are able to thermally relax, the rapid supply of energy causing them to rise above 100°C. This seems to be the case with the 350 ns heating of Greenwald *et al.*[2] and 1·5 μs heating of Nakagawa *et al.*,[4] since their histologies indicate the possibility of some steam generation causing the cell to rupture. In both of these cases the target vessels were normal sized and when a red blood cell ruptures it may rupture the vessel wall as well. Short time scales may not maintain the red blood cells of an ectactic vessel at an elevated temperature long enough for conduction of heat to the endothelial cells to cause thermal necrosis.

We conclude from the histologies that a 100 ms illumination time with 11–19 J/cm^2 of 578 nm light from a copper vapour laser pulsed at 15 kHz produces satisfactorily specific ectatic blood vessel necrosis.

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Clinical observations on delayed pressure urticaria

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SUMMARY

Repeated pressure tests at regular intervals were carried out on 48 patients with delayed pressure urticaria (DPU). Patients showed a considerable variation in response with periods of positive testing ranging from a few days to months or years. All the DPU patients had had chronic urticaria and/or angio-oedema although sometimes minor and occasional. Pressure tests were also carried out at regular intervals on seven patients with chronic urticaria who had weals at pressure sites but no history of pressure-induced weals (Köbner weals). These weals coincided with positive pressure tests and it seems likely that they all had DPU but only for a short time.

Delayed dermographism was studied in eight patients with DPU. There was a good correlation in time and degree between positive pressure tests and delayed dermographism, confirming that delayed dermographism is in fact DPU provoked by a slightly different mode of pressure.

Several aspects of delayed pressure urticaria (DPU) require a prolonged study of individual patients. Many of the tests devised to produce experimental weals in DPU are unsatisfactory for outpatients, including the usual method which is to press the dermographometer point hard onto the skin[1] and observe the results 4–6 h later. Recently a glass sphere, 1·5 cm diameter, has been used to concentrate the pressure on the outer aspect of the forearm beneath a cuff to which is attached a plastic bag containing various household materials to make up the approximate weight. The use and results of this sphere test have been described in detail[2] and is easy for patients to carry out, particularly if they are given the diagram that was published with the report. The test can be used by the patient 4–10 h before each out-patient appointment and so the condition of DPU can be studied on a regular basis.

METHODS

Survey (1)

Over the last 6 years 48 patients with DPU (females 16, males 32, average age 42, range 10–70 years) were studied. All patients had a clear history of typical DPU swellings occurring between

2 and 8 h after pressure. Sphere tests were carried out before each out-patient attendance at 2–3 week intervals and the results assessed by the author. Twenty patients also carried out tests twice per week for periods of 3–12 months, and six patients carried out the tests on a daily basis for periods of 3–6 weeks with the results assessed and charted by the patient. The site where the test was applied was not reused for at least 1 week. The test sites were examined 5–7 h after application and the results graded as large swellings (> 3 cm diameter), moderate swellings (1–3 cm diameter) and minor changes (red and palpable).

Survey (2)

Seven patients presenting with chronic urticaria were studied. They also had swellings at sites where pressure is likely, but they had no history of pressure preceding the development of the swellings. Sphere tests were carried out daily or at weekly intervals.

One of these patients who had severe urticaria and weals at pressure sites around the waist and on the shoulders was admitted and treated with a regular dose of antihistamines. Sphere tests were applied at 7·00 h, 12·00 h and 22·00 h every day for 3 days, care being taken to avoid the previously tested sites.

Survey (3)

In order to elicit delayed dermographism, eight patients with DPU were taught how to apply the dermographometer themselves. The tip of the obturator (metal slide) used in conjunction with the loaded point was pressed against the forearm by the underside of a table edge. The other hand then moved the dermographometer forward and backwards in the groove once; pressure 10 on the instrument scale was used being $5{\cdot}15 \times 10^5$ Pa. This was carried out on one forearm, and the sphere test applied to the other forearm, 5–7 h before being seen in the out-patient clinic. The procedure was continued for 2–3 months.

In four patients who were positive for both sphere tests and delayed dermographism tests, the dermographometer was applied at different pressures on the scale, namely 6 (0·9 Pa), 8 (3·1 Pa) and 10 (5·15 Pa).

RESULTS

Survey (1)

Thirty-eight patients had positive sphere tests on at least one occasion. Five of these were large swellings, 16 moderate swellings and 17 had minor changes. In the 10 patients who were repeatedly negative they were clinically improved by the time the test was carried out.

Length and variability of response. The repeated sphere tests remained positive for periods of a few days only (3–7 days) in six of the patients, a few weeks (1–8) in 11 patients and a few months (2–12) in 12 patients. Nine patients continued with positive sphere tests for periods of 1–6 years. In 18 patients, once the sphere test became negative it remained so, but other patients developed further periods when the test again became positive. The patients were usually aware of an increased tendency to DPU at these times.

The incidence of chronic urticaria and angio-oedema. Of the 48 patients with DPU, 28 also had phases of chronic urticaria together with angio-oedema and eight patients had angio-oedema alone. Twelve patients had very minor and very occasional attacks of chronic urticaria or angio-oedema playing little or no part in the overall symptomatology. If these relatively minor incidents are included, no cases of DPU were completely without an associated chronic urticaria or angio-oedema. In all cases the sphere test was more likely to be positive during a phase when chronic urticaria and/or angio-oedema were present, particularly if there was an acute episode of urticaria.

Survey (2)

Patients with chronic urticaria and swellings at pressure sites. Of the 7 patients with urticaria who also had some swellings at pressure sites, but no history of applied pressure, four patients had positive sphere tests with the usual timing of response and three were negative, but the tests in these three were carried out after the active phase of urticaria had died down. The pressure tests carried out every 5 h on the patient who was admitted were positive on most occasions for 2 days but negative on day 3 when the urticaria had improved and there were no more weals in pressure areas. Two weeks later the urticaria was clear and the pressure test was again negative.

Survey (3)

Delayed dermographism. Of the eight patients tested with the dermographometer 6 h before their out-patient attendance, six patients developed delayed dermographism and these were all associated with a positive sphere test on the other forearm. The two patients who gave no delayed response both had negative sphere tests at that time. On follow up of the six patients with both positive sphere tests and delayed dermographism both tests remained positive for 3–6 months in two patients. In four patients the sphere tests became negative after periods of 2–6 months and the delayed dermographism tests also became negative at the same time. Two of these patients later had exacerbations of their DPU association with the return of both the positive sphere tests and the delayed dermographic response. The four patients with both delayed dermographism and DPU who were tested to different pressures of 0·9, 3·1, and 5·15 Pa showed no delayed dermographism with pressure 0·9 Pa. Two patients showed delayed dermographism to 5·15 Pa only and two patients to 5·15 and 3·1 Pa. The latter patients showed large swellings on the sphere test at the same time.

DISCUSSION

The literature indicates that most DPU patients tend to have the condition for a prolonged period of time although the fluctuating nature of the disease has been mentioned.[3] It seems clear that many patients have phases when DPU is apparent and when the tests are positive. These phases may last only for a few days or many years and may recur at intervals, often coinciding with an increase of any chronic urticaria or angio-oedema. This variability of response may be responsible for negative tests when there has been a clear history of DPU.

In previous studies the incidence of patients with DPU with phases of chronic urticaria and angio-oedema have varied from 25% to 94%.[1,3–5] Some DPU swellings may not be associated with pressure by the patient and may be wrongly recorded as angio-oedema. Patients are also more likely to remember minor incidents of general weals or angio-oedema swellings after careful questioning, which have played little part in the overall symptomatology. In the present series it emerged that no patient had been completely without coincidental chronic urticaria or angio-oedema, though in some, this has been very minor and occasional. It would seem therefore that most patients with DPU also have, at times, chronic urticaria and angio-oedema.

The relationship of DPU to chronic urticaria seems analogous to the relationship of angio-oedema to chronic urticaria. Both DPU and angio-oedema are commonly associated with chronic urticaria and are worse during phases when the urticaria is more troublesome. However, in a few subjects the condition is predominently DPU or angio-oedema and presents with very minor chronic urticaria or none at all.

In phases of acute and chronic urticaria, swellings may develop at sites of pressure, but without a history of pressure. These have been regarded as being distinct from DPU and are referred to as Köbner weals.[6,7] The present study indicates that pressure tests carried out at the time when these swellings develop are positive after the usual time interval so characteristic of DPU. Furthermore it has been shown that DPU swellings are more likely to occur when any coincidental chronic urticaria is more active. Pressure tests in patients with urticaria but without weals at pressure sites have always been negative.

It may be argued that in patients described as having Köbner weals, the type of pressure causing the weals is slightly different from that commonly reported as causing DPU swellings. For example, areas of tight clothing are likely to be the sites of Köbner weals whereas in ordinary DPU swellings may develop at pressure sites from standing on a ladder, using tools and carrying heavy objects. However, this disparity may be due to the fact that patients who are having an attack of urticaria are much less likely to be indulging in heavy work. Certainly in ordinary DPU swellings will readily develop at sites of pressure from clothing. It does therefore seem likely that what have been described as Köbner weals and considered as a separate entity are in fact due to a short-lived phase of DPU.

Delayed dermographism has been considered as a separate entity from, but commonly associated with DPU. In a recent report 55% of 29 DPU patients tested had delayed dermographism.[1] In the present study there was a complete correlation between the development of delayed dermographism and positive DPU sphere tests and this was particularly noted in the follow up of the patients. The delayed dermographism lesions at the test site always had a similar time course and clinical appearance (except for linearity) as the lesions of pressure urticaria. Furthermore it was shown that in order to cause delayed dermographism it was necessary to exert a high pressure similar to that required to provoke DPU. It seems that delayed dermographism is DPU provoked by a slightly different mode of pressure.

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Preventive effect of an *E. coli*-filtrate (Colibiogen®) in polymorphous light eruption

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SUMMARY

During the administration of Colibiogen®, a commercially available *E. coli*-filtrate, there was a significant ($P<0{\cdot}01$) increase of the test dose of UVA necessary to elicit skin lesions in nine patients with polymorphous light eruptions. Control tests performed in the same manner without the drug indicate that this was due to treatment. Colibiogen® appears to be of value in the treatment of polymorphous light eruption (PLE).

Various therapeutic modalities are in use for the treatment of polymorphous light eruption (PLE). Besides sun avoidance and the application of appropriate sunscreens, photochemotherapy[1–5] or UVB-therapy[4–6] are successful treatments. Antimalarials,[7–11] carotenoids[2,9,10,12–14] and nicotinamide[15] have also been recommended.

All these prophylactic measures apart from sun avoidance are empirical. We were interested to hear about a female suffering from long-standing PLE who became free of her photodermatosis while on Colibiogen® for an unrelated condition. Colibiogen®, which is a protein-free filtrate derived from cultures of *E. coli*, has been available in Germany for many years and used in the treatment of gastrointestinal and allergic conditions. The observation that it improved a patient with PLE led us to investigate its efficacy in others with this condition.

METHODS

Patients

Nine patients with the papular type of PLE (three males, six females; aged 21–47 years; suffering from PLE for 3–19 years) were studied. The diagnosis of PLE had been established by a characteristic clinical history and by previous positive results at phototesting with histologic examination of skin lesions.[16] Other UV-induced dermatoses had been excluded by appropriate

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laboratory tests (direct immunofluorescence examination of skin biopsies, determination of antinuclear and anti-Ro-antibodies and screening for porphyrins).

Irradiation sources

UVA was provided by an UVASUN 5000 apparatus (Mutzhas, Munich, F.R.G.) emitting in the range of 320–460 nm (maximum approximately 375 nm).

For UVB irradiations, TL20W/12 light bulbs (Philips, Hamburg, F.R.G.) with main emission spectrum between 275 and 365 nm (maximum at 315 nm) were used. Irradiance at a distance of 40 cm, at which tests were performed, was about 47 mW/cm^2 for UVA and 1·0 mW/cm^2 for UVB. UVA or UVB were measured by an integrating instrument (Centra-UV-Meßgerät; Osram, Munich, F.R.G.).

Test drug

Colibiogen® (Laves, Ronnenberg/Hannover, F.R.G.), a filtrate of cultures of *E. coli* free of bacteria and protein, contains amino acids, peptides, polysaccharides and fatty acids. One millilitre of the commercial product comprises the filtrate of about 2 × 10^{12} *E. coli* bacteria.

Test procedure

On 3 consecutive days (0, 1, 2) incremental doses of 10, 20, 40 and 80 J/cm^2 UVA were given to distinct test areas, measuring 5 × 5 cm each, on the extensor side of the right upper arm. In two patients all test areas were exposed additionally to low UVB doses (120 mJ/cm^2 in total), as this had previously been found to enhance the development of skin lesions. Colibiogen® was started on day 7. Seven patients were given 2 ml intramuscularly 3 times a week, and two received 5 ml orally t.d.s. The UV test procedure described above was repeated on days 21–23. The drug was then discontinued. Control testing, carried out according to the same schedule without Colibiogen®, was performed in four patients who had not taken the drug for at least 8 months.

Evaluation of test results

Test areas were evaluated for the presence of erythema or papules 24 h following each irradiation (days 1, 2, 3, 22, 23, 24) and before the next UV dose was applied. The cumulative threshold doses for the elicitation of skin lesions were calculated as the lowest sum of UVA doses necessary to provoke a reaction in any of the four test areas.

RESULTS

Before treatment the cumulative threshold doses for the elicitation of any skin lesions were 20–120 (median 60) J/cm^2 UVA. During administration of the drug they increased to 30–> 240 (median 120) J/cm^2 ($P < 0{\cdot}01$; Wilcoxon matched pairs signed rank test) (Fig. 1). The cumulative threshold doses for the elicitation of papules increased from 30 to > 240 (median 80) J/cm^2 UVA to 80–> 240 (median 240) J/cm^2 ($P < 0{\cdot}01$; Wilcoxon matched pairs signed rank test) (Fig. 2).

With the controls (Fig. 3) the cumulative threshold dose for elicitation of any kind of skin lesions showed no change in two, was increased in one and decreased in another of the four patients. Thus, reduced photosensitivity occurred significantly less frequently than in the treatment group ($P < 0{\cdot}05$; Chi-square test). No increase of UV tolerance was found with respect to the elicitation of papules in any of three patients. One individual did not produce papules.

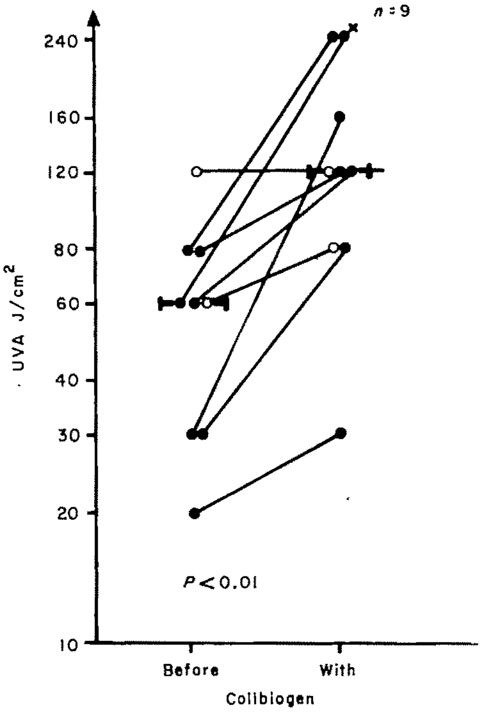

FIGURE 1. Cumulative threshold doses for the elicitation of any skin lesions before and during treatment with Colibiogen®. ●, intramuscularly; ○ orally; *x*, >240 J/cm^2; ——, median.

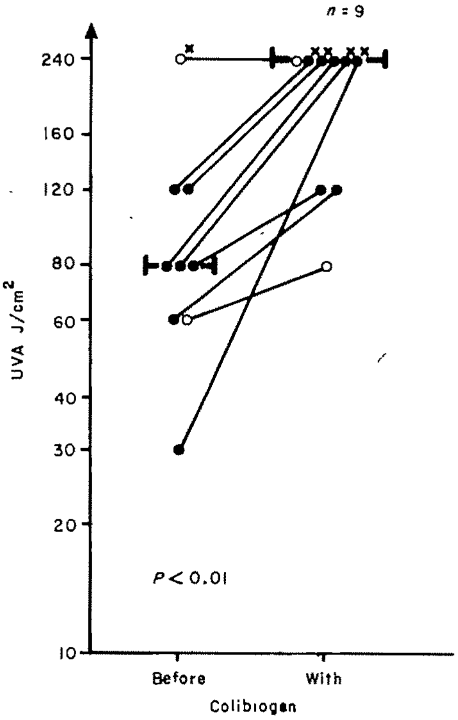

FIGURE 2. Cumulative threshold doses for the elicitation of papules before and during treatment with Colibiogen®. (Symbols as for Figure 1).

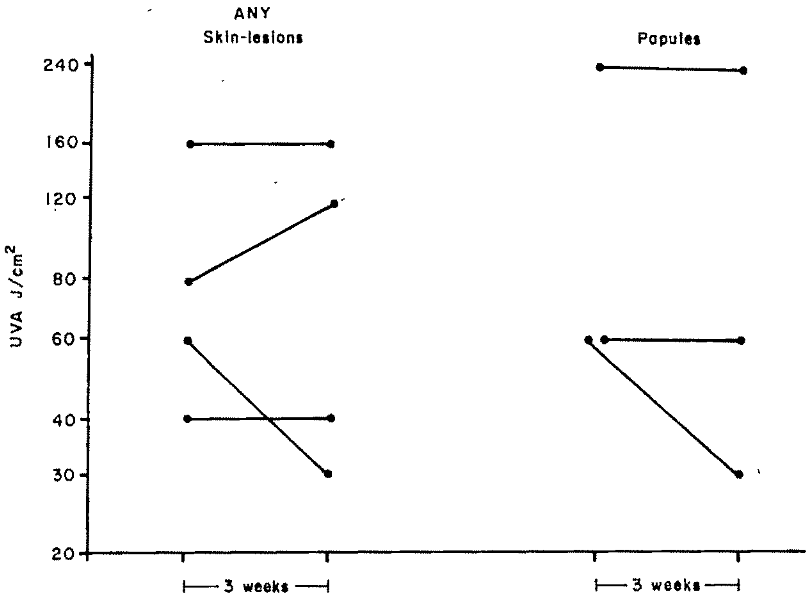

FIGURE 3. Control testing: cumulative threshold doses for the elicitation of any skin lesions or of papules.

DISCUSSION

In patients with PLE, Colibiogen® treatment resulted in an increase of the UV dose needed to produce skin lesions. While the development of papules can be regarded as a reaction specific for PLE, erythema may represent initial lesions of PLE, a toxic skin response to higher UV doses, or a combination of both. The development of papules or of any visible pathologic skin lesions (erythema and/or papules) was therefore evaluated separately. Increase of UV tolerance was found for both parameters, treatment being somewhat more effective with regard to the elicitation of papules. Although the number of individuals available for control testing was small, the change in reactivity to UV as seen with the treatment using Colibiogen® in our patients was considered as not having occurred spontaneously. Photo-provocation threshold testing as performed here seems to be a valuable method to obtain information on the clinical efficacy of therapeutic regimens in PLE.

It is not known by what mechanism Colibiogen® reduces the reactivity of the skin to UV light. Investigations performed more than 30 years ago showed that Colibiogen® inhibited the haemolysis of human erythrocytes by bacteria or from osmotic effects and enhanced the oxygen consumption of human skin cells.[17] Later studies have indicated that, in experimental conditions, it has a preventive effect against infection.[18] Preliminary data (unpublished) suggest an inhibitory effect on anti-IgE-induced histamine release from rat mast cells. Colibiogen® appears to have a protective effect on mammalian cells and may be regarded as a biologic response modifier.

Our data suggest that administration of Colibiogen® may be of value in the prophylactic treatment of PLE, but the clinical efficacy of the drug has yet to be determined. A double-blind clinical trial of this preparation is currently in progress.

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Cholinergic pruritus, erythema and urticaria: a disease spectrum responding to danazol

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SUMMARY

Cholinergic pruritus and cholinergic erythema are relatively rare conditions believed to represent variants of cholinergic urticaria. We report an extremely severe and unusual case demonstrating features of both these variants before evolving into more classical cholinergic urticaria. This strongly supports the view that all three syndromes are variations of the same disease. This case demonstrated a dramatic symptomatic response to danazol which has not previously been reported.

CASE REPORT

A 19-year-old male presented to the Leicester Royal Infirmary in November 1987 with a 3-year history of increasingly severe pruritus. This occurred in episodes which were initially induced only by physical exertion but later became more frequent and were triggered by being in a warm room, by anxiety and hot food. The symptoms were alleviated by going to a cool place for a few minutes, typically resolving after 5 min in suitable surroundings. The pruritus was so severe when present that he was unable to maintain concentration on anything and had to give up attending college. At no time did he complain of a rash. The pruritus responded only transiently to promethazine, initially prescribed by his general practitioner and he subsequently failed to respond to chlorpheniramine, brompheniramine, terfenadine and ibuprofen.

A careful examination at presentation revealed no abnormality with no skin lesions even when the pruritus was induced by physical exertion.

Over the course of the next 6 months the pruritus continued to increase in severity in spite of treatment with brompheniramine 4 mg t.d.s., terfenadine 120 mg b.d. in combination with cimetidine 200 mg t.d.s., aspirin 600 mg daily, paracetamol 1 g t.d.s., and piroxicam 20 mg daily. No useful benefit was obtained by provoking attacks in the hope of inducing a subsequent period of respite from symptoms. It was noticed at follow-up visits that a mild erythema was occasionally present over the shoulders and this gradually became a more prominent feature. A further exercise challenge was performed in May 1988 and on this occasion we observed two small papules typical of cholinergic urticaria on the chest.

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Results of initial investigations, including routine biochemistry and haematology, thyroid function and serum ferritin were all normal. In April 1988 the plasma anti-protease levels were assayed: α1-antitrypsin 1·8 g/l (1·1–2·1), C1q esterase inhibitor 0·14 g/l (0·15–0·35), α1-antichymotrypsin 0·21 g/l (0·3–0·6). In view of the low α1-antichymotrypsin level it was decided to perform a trial of treatment with danazol 200 mg t.d.s. After 2 weeks on danazol and with no other treatment, the pruritus almost totally resolved. Five months later he remained virtually asymptomatic. The level of α1-antichymotrypsin was reassayed 2 months after commencing danazol and had risen to 0·75 g/l.

DISCUSSION

The presenting features in this patient were very similar to those of three patients reported by Nomland in 1944,[1] who termed the syndrome 'cholinergic itching'. The inducing factors appeared identical to those inducing cholinergic urticaria, which had then only recently been described. Nomland's patients also were severely handicapped by episodes of intense pruritus induced by heat, exercise, hot food or emotion and relieved by cooling the skin. One of these patients was considerably embarrassed by a compulsion to take his clothes off and dowse himself in cold water to relieve attacks, which would often occur in restaurants. These patients were regarded as suffering from a variant of cholinergic urticaria.

There has subsequently been little in the literature about this condition, except for a study which demonstrated low levels of cholinesterase in the skin of two patients with cholinergic pruritus.[2] It is interesting that the study also showed rather low levels of this enzyme in the skin of patients with cholinergic urticaria.[2]

Another, apparently similar condition termed 'cholinergic erythema' has recently been described.[3] In this condition, also, patients complain of pruritus induced or exacerbated by exercise and warmth. However, this is accompanied by visible skin changes consisting of a macular punctate erythema, sometimes with generalized erythema. The authors regarded this as another variant of cholinergic urticaria.

The pattern of illness in our patient has always been very severe, and the pruritus has been very incapacitating. However, it has apparently changed in nature from a presentation with cholinergic pruritus with no accompanying rash, through a phase compatible with the newly described entity cholinergic erythema and finally (with the development of papules on exercise testing), to cholinergic urticaria. This strongly supports the view that these three conditions represent a spectrum of the same disorder.

It has been demonstrated that cholinergic urticaria is associated with low plasma levels of α1-antichymotrypsin[4] and it has been postulated that this promotes the inflammation by delaying inactivation of inflammatory proteases. In another recent study a therapeutic response of cholinergic urticaria to danazol has been demonstrated,[5] and this was accompanied by a correction of low α1-antichymotrypsin levels. This response was recorded as a reduction in the number of urticarial papules induced by stress testing but the authors were not able to confirm symptomatic improvement. We believe this is the first time that a dramatic symptomatic improvement has been reported in response to danazol.

The improvement in the plasma level of α1-antichymotrypsin in our patient (from below to above the normal range) would support the view that a low level of this enzyme is of significance in cholinergic urticaria. However, the persistence of occasional relatively mild pruritus in spite of adequate correction of the level of this enzyme suggests that there may also be other factors involved. It is possible that the low level of skin cholinesterase, previously described in this group of disorders,[2] is a further predisposing factor.

Unfortunately, danazol, in common with other C17-alkylated steroids, may induce cholestatic hepatitis.[6,7] Although this is usually reversible, it is essential to monitor liver function during treatment. Care should also be taken to ensure that danazol is never given during pregnancy, as it has a virilizing action on the female foetus. Cholinergic urticaria is not a licensed indication for danazol. It is therefore recommended that the use of this drug should be reserved for very severe cases of cholinergic pruritus, in which a low level of α1-antichymotrypsin has been demonstrated.

ACKNOWLEDGMENTS

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Multiple minute palmar-plantar digitate hyperkeratoses

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SUMMARY

Digitate hyperkeratoses are rare lesions of unknown cause. We wish to add a new variant of this entity to the few reports in the literature. Our patient is a 79-year-old woman with palmar pits and digitate hyperkeratoses limited to her palms and soles.

The first case of multiple minute digitate hyperkeratoses was reported by Goldstein in 1967.[1] To date there are a total of nine reports of 13 cases of this entity also known as disseminated spiked hyperkeratosis, familial piliform disseminate hyperkeratosis, porokeratotic minute digitate hyperkeratoses, and minute aggregate keratoses.[1-9] We report a new variant of this condition in an elderly woman with lesions limited to the palms and soles and associated with palmar pits.

CASE REPORT

A 79-year-old black woman presented to the dermatology clinic of Columbia-Presbyterian Medical Center with a 6 month history of asymptomatic lesions on the plantar aspect of both feet. The patient could not relate the appearance of these lesions to any particular event. She denied any known prior exposure to arsenic, although she did drink well water as a young woman.

Her past medical history included hypertension, adult-onset diabetes mellitus, congestive heart failure, peripheral vascular disease, and arthritis. She had had a myocardial infarction, appendectomy, total abdominal hysterectomy, and right femoral and popliteal angioplasty. Her medications included dypridamole, digoxin, hydrochlorothiazide, sucralphate, acetaminophen, and multivitamins. She denied alcohol intake and cigarette smoking. Her family history was not known.

On physical examination she had approximately 15–20 filiform papules scattered on the soles of both feet, and one isolated papule on her left palm. The lesions were about 1 mm in diameter

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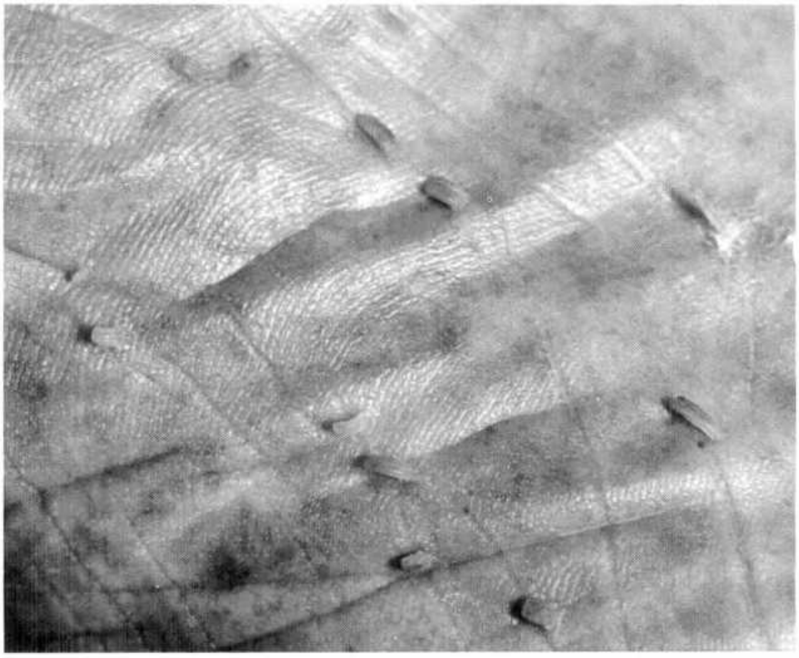

FIGURE 1. Multiple minute digitate hyperkeratoses on the sole of the foot.

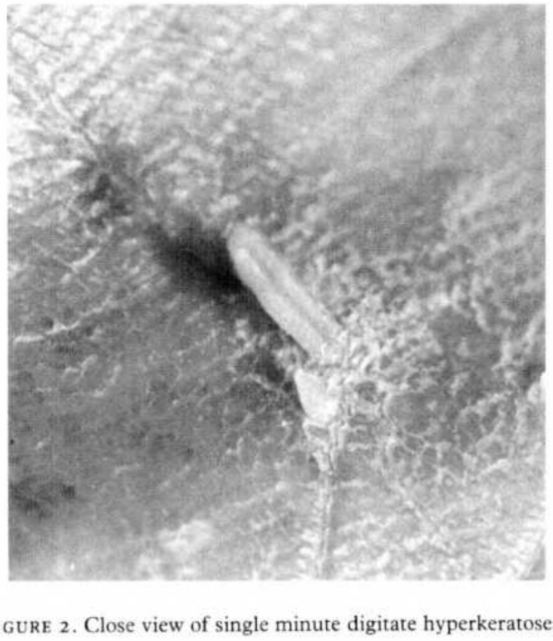

FIGURE 2. Close view of single minute digitate hyperkeratoses.

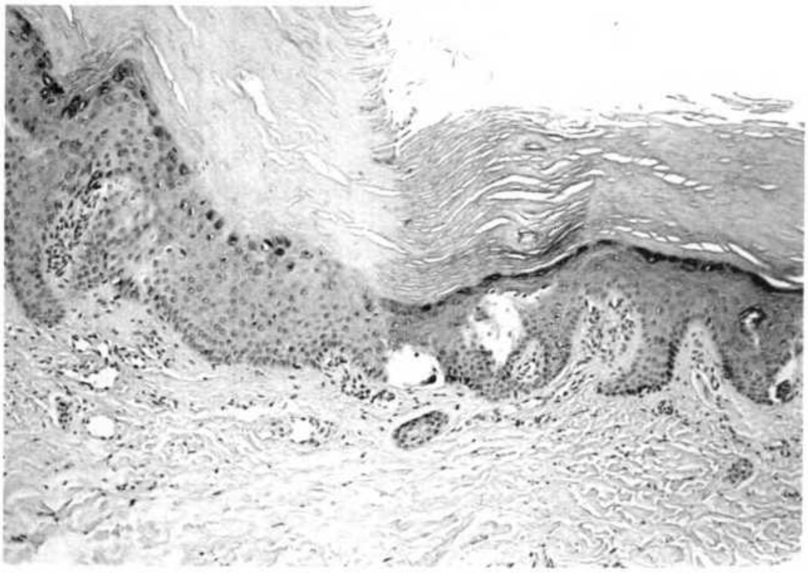

FIGURE 3. Histologically minute digitate keratoses show sharply circumscribed, generally orthokeratotic hyperkeratosis arising from a focus of acanthosis.

and 1–3 mm in height (Figs 1 and 2). A potassium hydroxide preparation was negative. She also had two pits 1 mm in diameter on each palm sparing the palmar creases.

The histology of one of the papules showed a focal area of marked hyperkeratosis overlying digitate malpighian hyperplasia. There was lack of the normal flattening of the keratinocytes in the upper layer of the epidermis. There were focal areas that lacked a granular layer which corresponded to rare retained parakeratotic nuclei within the overlying thickened generally orthokeratotic horn. A periodic acid-Schiff stain for fungus was negative. In the dermis there were a few ectatic blood-vessels (Fig. 3).

DISCUSSION

In 1967 Goldstein reported the first case of multiple minute digitate hyperkeratoses in a 32-year-old soldier with a 5-year history of skin-coloured, nonfollicular, projectile papules on the chest, arms, and legs.[1] The past medical history included peptic ulcer disease and a kidney infection, neither of which appeared to be related to these lesions. Histologically the lesions demonstrated focal hyperkeratoses with mild acathosis and elongation of the rete ridges. Several areas showed thickening of the granular layer and a few vacuolated cells. Most recently similar lesions were reported in a 48-year-old woman who, since the age of 20, had multiple small keratotic lesions on her chest, back, and abdomen.[2] Her past medical history included breast surgery for fibrocystic disease, a hysterectomy for uterine fibroids, and hypertension. Histology revealed focal hyperorthokeratotic horn covering two to six elongated rete ridges, a normal or decreased granular layer, mild acanthosis, and a mild perivascular infiltrate.

In Nedwich and Sullivan's review of the literature,[3] it is clear that cases reported as disseminated spiked hyperkeratosis[4] and familial piliform disseminated hyperkeratosis[5] were probably all examples of the same entity first reported by Goldstein under the name of multiple minute digitate hyperkeratoses. Common to these cases were: (i) asymptomatic, projectile papules on the trunk and extremities with sparing of the palms and soles; (ii) onset in early adulthood; (iii) equal distribution among males and females; (iv) apparent autosomal dominant inheritance in six of the reported cases; (v) no apparent association with a particular disease (except the patient reported by Ferrandiz *et al.*[6] whose lesions resolved after surgical treatment of a carcinoma of the larynx) or with any medication; (vi) focal hyperkeratosis and acanthosis with a relatively normal dermis; (vii) transient moderate response to emollients and keratolytics.

In 1987 Aloi *et al.* reported a 38-year-old woman with a 1-year history of asymptomatic, nonfollicular 'filiform horn projections' on her chest, shoulders, and arms.[7] She had a history of Crohn's disease and her medications included salicylazosulphapyridine, metronidazole, and corticosteroids. Histology of the skin lesions revealed a plug of parakeratotic horn emanating from an epidermal invagination. Some of the keratinocytes showed vacuolar degeneration. This condition was described as porokeratotic minute digitate hyperkeratoses. Despite some of the differences in the histologic features of these lesions, the clinical similarity would place them in the entity of multiple minute digitate hyperkeratoses.

Our patient had asymptomatic projectile papules which erupted without any obvious cause and on histology showed marked focal orthokeratotic horn. However, unlike the other reported cases, her lesions arose at a much later age and were limited to her palms and soles with a few palmar pits. The focal lack of a granular layer and abnormal maturation of the keratinocytes is also unique to this patient. We believe these lesions to represent yet another variant of multiple minute digitate hyperkeratoses.

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Anti-SL-Ki antibody in a patient with fatal connective tissue overlap disease

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SUMMARY

A patient with photosensitivity is described who presented with an overlap syndrome combining features of systemic lupus erythematosus with a mild polymyositis and scleroderma and with symptoms of the sicca syndrome. In addition, he was anti-SSA/Ro positive and had a constellation of antibodies directed to ENA including the anti-SL-Ki antibody. The condition was fatal and he died from heart failure and respiratory insufficiency.

The overlap syndromes are entities in which there are features of more than two connective tissue diseases occurring in the same patient. A classification of such syndromes has not yet been established, but different precipitating antigen-antibody systems have been identified that seem to characterize some of these disorders. An antibody directed to a ribonucleoprotein complex (U1-RNP) belonging to a saline extractable nuclear antigen complex (ENA) is commonly found in the mixed connective tissue disease (MCTD), and an antibody directed to another ENA, the Ku antigen, points to the scleroderma-polymyositis (PSS-PM) overlap syndrome. Another anti-ENA antibody, anti-SL-Ki, has been recognized as defining systemic lupus erythematosus (SLE) in patients who often have anti-Sm antibodies and have arthritis, pericarditis, pulmonary hypertension and the sicca syndrome.[1,2] The dermatological features are those of SLE and include photosensitivity.[3] Sclerodermatous features have been reported only in one anti-SL-Ki-positive patient who was diagnosed as having MCTD,[3] though they can be found in patients with anti-Ku antibodies which have some similarity with anti-SL-Ki.[4]

CASE REPORT

A 67-year-old man with a 20-year-old history of pulmonary disease presented in 1982 with symptoms of Raynaud's phenomenon and photosensitivity. He also had arthralgias that dated from 1965, and in 1978 was diagnosed as having mitral stenosis with insufficiency. He had dysphagia and complained of a dry mouth.

On examination, there was some periorbital oedema with marked telangiectasia of the skin on

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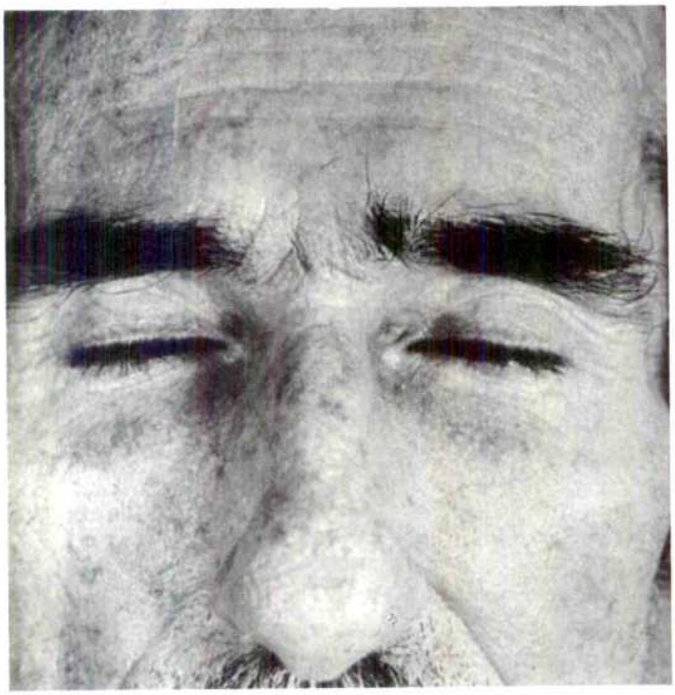

FIGURE 1. Telangiectases of the face.

his face (Fig. 1) as well as on the trunk and fingers. There were numerous oval hypochromic macules on the back and chest and there was a diffuse brown hyperpigmentation of the skin. Sclerodactyly was present affecting the fingers and toes, with some loss of hair on the backs of the fingers and toes.

Laboratory investigations revealed an elevated blood sedimentation rate (ESR), being 90 mm/h. There was slight proteinuria and haemoglobinuria. Blood urea and creatinine were moderately elevated and the creatinine clearance was reduced (37 ml/min). Serum transaminases were markedly elevated. The gamma globulins were raised (39·1 g/dl) with an elevated IgG. The rheumatoid factor was positive 1/320 and there were circulating immune complexes. The level of C3 was reduced being 50 mg% (normal 75–100) and the C4 level was 18 mg% (normal 30–40). Chest X-rays showed interstitial fibrosis, and pulmonary function studies indicated pulmonary fibrosis. A barium swallow examination resulted in reflux into the trachea.

A skin biopsy was taken from the abdomen and showed typical features of scleroderma (Fig. 2). Direct immunofluorescence of both involved and uninvolved skin showed speckled deposits of IgG in the epidermal nuclei (Fig. 3) and deposits of C3 in the vessels of the upper dermis. In the serum a circulating speckle patterned IgG was present in a titre of 1 : 640 when the serum was tested on both rat liver and KB cell substrates. Immunodiffusion revealed antibodies to anti-SL-Ki (> 1/100) (courtesy of E.M.Tan, Scripps Clinic, La Jolla, CA, U.S.A.), but antibodies to Sm antigen were absent. Counter-immunoelectrophoresis (CIE) detected antibodies directed to SSB/La and SSA/Ro antigens (1/32 and 1/128 respectively).

The patient was on prednisolone for his respiratory problem and this treatment was not modified. He was again seen 3 years later with a bullous eruption on the trunk and limbs that was considered to be erythema multiforme, probably due to him taking trimethoprim-sulphameth-

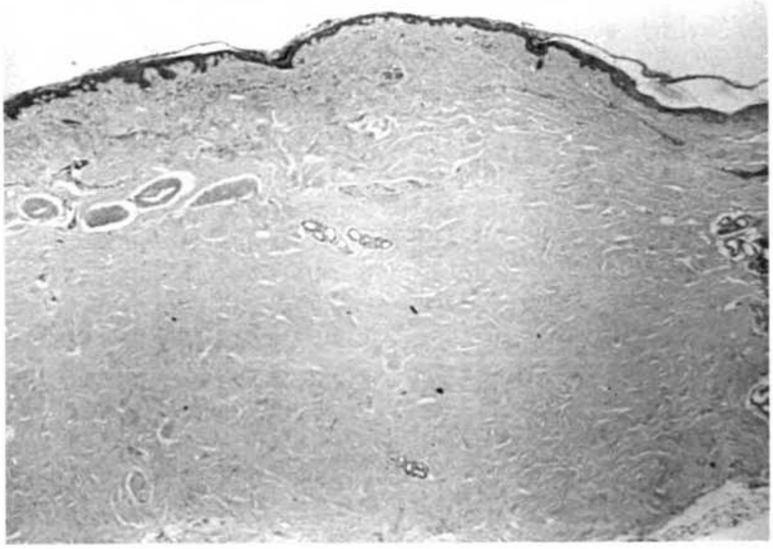

FIGURE 2. Typical sclerodermatous changes in the skin.

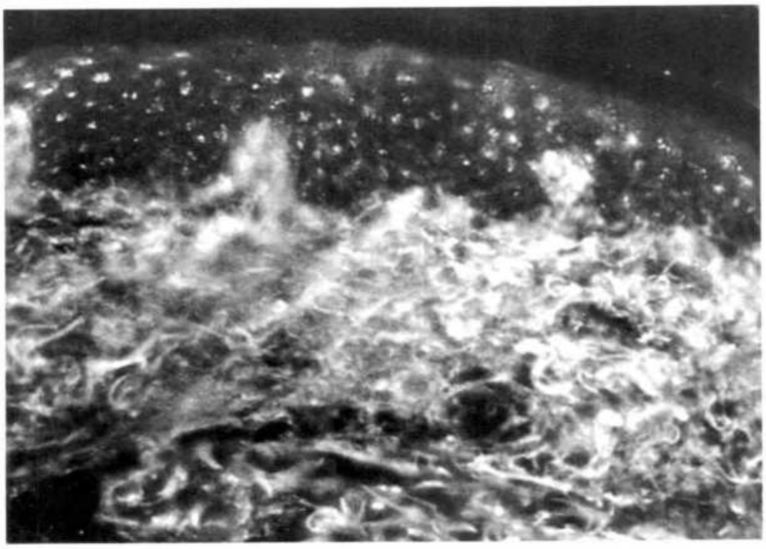

FIGURE 3. IgG in the nuclei of keratinocytes (DIF).

oxazole. Laboratory investigations performed at that time showed very little change, though the creatinine clearance was further reduced, being 23 ml/min.

In September 1985 his condition deteriorated and in November he died from heart failure and acute respiratory insufficiency.

DISCUSSION

In our patient there were signs of four connective tissue disorders. The non-erosive arthritis, the photosensitivity with anti-SSA antibodies with renal involvement and low complement levels all suggest systemic lupus erythematosus. Rowell's syndrome may have been suspected when there was the EM-like eruption, but this was probably due to previous sulphonamide therapy.[5] The periorbital oedema associated with the increase in the serum transaminases could account for polymyositis, though it is likely that the elevated enzyme levels were nonspecific. The skin and other organ involvement were those seen in scleroderma, but in addition our patient had the symptoms and the serology of the 'sicca' syndrome.

Some of the clinical features shown by our patient were those of mixed connective tissue disorder (MCTD), in particular the modest sclerodactyly, the Raynaud's phenomenon and the presence of antibodies directed to the epidermal nuclei as seen on direct immunofluorescence. Other features however are rarely encountered in MCTD, such as photosensitivity and in addition, in our patient the peculiar serology was not consistent with that classically found in MCTD. Antinuclear antibodies were not present in a high titre.[6] A number of anti-ENA antibodies were detected, and anti-Sm and anti-Ku were absent, but there were present anti-SSA/Ro, anti-SSB/La and anti-SL-Ki antibodies.

ENA is an antigenic complex which includes two major and several minor antigenic systems. The two major systems are the Sm and U1 and RNP antigens, suggestive of SLE and MCTD respectively. In laboratory observations Sm antigen is RNAase-resistant, whereas U1-RNP is usually considered as corresponding to an RNAase sensitive component of ENA. Among the minor systems, SL-Ki, Ku (p70/p80), Su and SSB/La antigens are RNAase-resistant.

The SL-Ki antigens are non-histone, DNAase and RNAase resistant and trypsin-sensitive proteins. Approximately 10% of patients with SLE produce an antibody frequently associated with anti-SM.[1] The anti-SL-Ki antibody can be subclassified into three classes according to its capacity to react with two different SL-Ki epitopes of 66 kDa and 86 kDa respectively. Class I antibodies react with both epitopes, while class II antibodies react only with the 66 kDa epitope and class III only with the 86 kDa epitope.[4] In our patient anti-SL-Ki was not subtyped.

In 1985, Reeves *et al.* found that six out of 15 patients with scleroderma had circulating antibodies to a p70/p80 antigen.[7] The SL-Ki system was later said to be immunologically identical to the p70/p80 system[4] which is in turn identical to Ku. However, the clinical features associated with these systems are different and no anti-Ki antibodies were detected in the sera of about 100 scleroderma patients.[4] The patient, therefore, is unusual in that he had sclerodermatous features with anti-SL-Ki positive antibody.

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Skin pigmentation associated with minocycline therapy

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SUMMARY

A patient on long-term minocycline therapy developed blue-black discoloration on the legs. Skin biopsy specimens from the pigmented areas were examined by light and electron microscopy and energy-dispersive X-ray microanalysis. Pigmented granules were present at all levels of the dermis and subcutaneous fat tissues. Ultrastructural examination showed electron-dense granular material within the cytoplasm of dermal macrophages and energy-dispersive X-ray microanalysis indicated that the granules contained iron. Thyroid tissue obtained by aspiration biopsy showed the presence of fine brown granules within the cytoplasm of the follicular epithelial cells. An extract of skin from the pigmented areas was subjected to high performance liquid chromatography and minocycline was detected.

Minocycline is a broad-spectrum antibiotic of the tetracycline family and was introduced in 1967. Since then, it has been widely used for treatment of infections and in patients with acne. Even though reported side-effects are few, long-term and high-dose administration of minocycline has caused pigmentation of the skin,[1–7] nails, teeth[8,9] and thyroid gland.[10–14] In 1972, a case was reported with transient hyperpigmented macules on the legs that was associated with long-term minocycline treatment.[15] Several histochemical, electron microscopic and electron X-ray analytical studies have been carried out to determine the nature of the pigment in the lesional skin, but this is still unclear.[1–4]

We report a case with marked blue-black discoloration of the skin who had received long-term minocycline treatment. We present the findings on light and electron microscopy and energy-dispersive X-ray microanalysis on a skin biopsy from the hyperpigmented skin of our patient. We also have found and identified minocycline by high performance liquid chromatography (HPLC) in extracts of the lesional hyperpigmented skin.

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CASE REPORT

A 53-year-old woman was first seen at the Dermatology Clinic of Osaka University Hospital in December 1987 with blue-black pigmentation of the skin on both legs. She had a 10-year history of varicose veins in both legs with dependent oedema. She had the varicose veins stripped and following the operation the sites became eroded, and she had a persistent exudative dermatitis with evidence of infection. The patient was started on minocycline (200 mg/day) in May 1986 and 6 months later she noted black pigmentation of the skin on the scars on her legs from the previous operation. Over the following months other areas of macular blue-black pigmentation appeared which extended on both legs and on the dorsum of the feet. The patient was otherwise in good health and had taken no other drugs over this period. In December 1987, the minocycline was stopped. The total dose was 114 g.

On physical examination there was an extensive area of blue-black pigmentation on the anterior aspects of both legs (Fig. 1). The pigmentation was particularly prominent at the scarred areas from the previous operation. No discoloration was noted of the fingernails or teeth. The results of laboratory investigations that included a full blood count, urinalysis and blood chemistry were all within the normal range. The thyroid of the patient was not enlarged and the thyroid function tests were within the normal range.

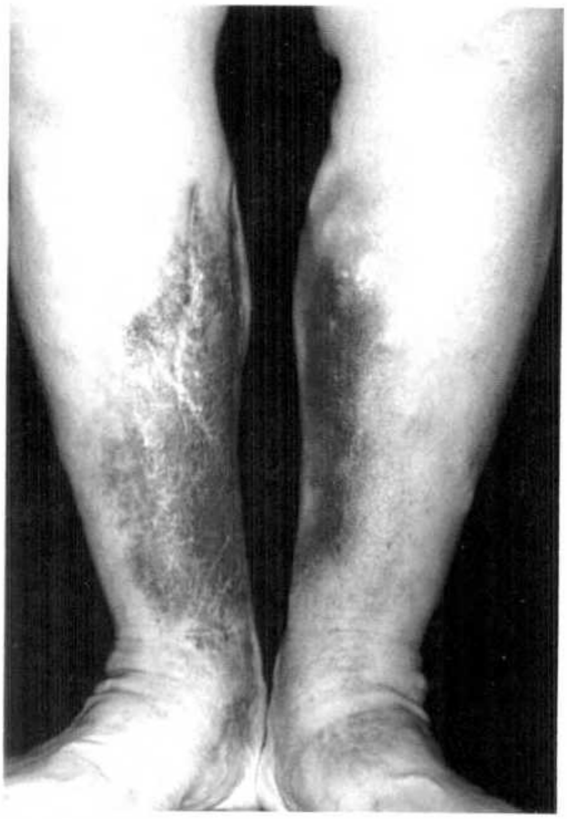

FIGURE 1. Blue-black pigmentation on the anterior aspects of both legs.

METHODS

Light microscopy

Skin biopsy specimens were taken from heavily pigmented areas on the right leg. The specimens were fixed in 10% formalin, embedded in paraffin, and sections stained with haematoxylin and eosin, and with the Prussian blue method for iron and the Fontana Masson method for melanin.

Electron microscopy

Skin specimens were cut into 1 mm^3 portions and fixed in 2·5% glutaraldehyde. After washing in phosphate-buffered saline, the specimens were post-fixed in 1% osmium tetroxide and then dehydrated through a graded series of alcohol and embedded in epoxy resin (Epon 812). Ultra-thin sections (80 nm) were stained with uranyl acetate and lead citrate and examined in a transmission electron microscope (Hitachi HU-12).

X-ray microanalysis

For X-ray microanalysis, 200 nm sections of the skin specimens were taken. Unstained thin sections were mounted on unsupported 200-mesh copper grids and then coated with carbon. The analytical electron microscope was a JEOL-100CX equipped with an energy-dispersive ORTEC EEDS II X-ray spectrometer system. Electron-dense bodies and background areas of cytoplasm were selected using a fine spot 120 nm in diameter. The metallic distribution pattern over selected fields was surveyed and the data recorded on film.

Aspiration biopsy of thyroid tissues

An aspiration biopsy of the thyroid was taken and smears of the tissue were prepared on glass slides, fixed with ethanol and stained.

Analysis of minocycline in skin tissues

The analysis of minocycline in the skin was carried out in the laboratory of Japan Lederle Ltd. Skin samples obtained from the discoloured area were homogenized in 1 M $MgCl^2$-ethanol solutions (pH 5·0) and left at room temperature for 48 h and then centrifuged at 4000 *g* for 5 min. The supernatant was air-dried and dissolved in distilled water, and then subjected to high performance liquid chromatography (HPLC) (Shimadzu LC-6A). Minocycline in the same solvent was also subjected to HPLC and chlortetracycline was used for an internal standard. The HPLC system consisted of a Shimadzu LC-6A with an SPD-6A absorbance detector and the wavelength used was 353 nm. The column was Partisil-10 ODS (300 mm × 4·6 mm). The mobile phase consisted of 0·1 M citric acid 0·1 M ammonium citrate in 20% DMF (pH 4·1), which was pumped at a flow rate of 1·3 ml/min at room temperature.

RESULTS

Light microscopy

Numerous dense deposits of brown-black pigment were apparent throughout the dermis and subcutaneous fat (Fig. 2). The pigment was distributed in various sized aggregates and were Prussian blue positive staining for iron. The Fontana Masson stain was negative apart from the melanin in the epidermis which appeared to be increased. A few mononuclear cells were observed in the upper dermis. Extravascular leakage of erythrocytes was not evident and there was only a slight pigmentary incontinence in the upper dermis.

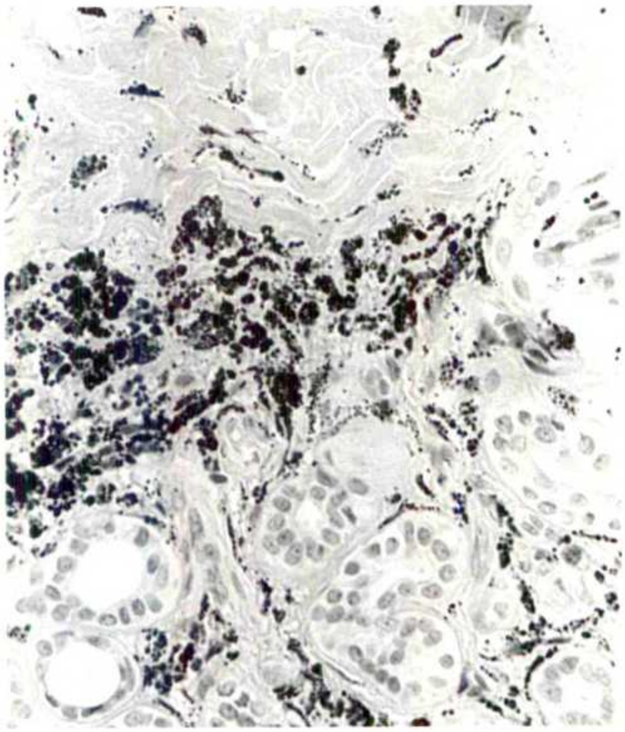

FIGURE 2. Light microscopy shows dense deposits of brown-black pigment in the dermis (haematoxylin-eosin, × 320).

Electron microscopy

This revealed dermal macrophages that contained variable aggregates of electron-dense granular material within their cytoplasm (Fig. 3). Some of these aggregates measured up to 2 μm in diameter and were not surrounded by an outer limiting membrane. They were not found extracellularly.

X-ray microanalysis

The spectral emission lines for the electron-dense granules seen in the dermal macrophages demonstrated high peaks for iron and microanalysis over the other areas in the cytoplasm showed no significant iron peaks (Fig. 4). The X-ray spectrograms showed low peaks of osmium and chloride as well as prominent peaks of copper. These were ascribed to the chemicals used in the fixative and embedding medium and to the copper grids supporting the thin sections.

Light microscopy of thyroid smears

A few clusters of follicular epithelial cells were obtained. Some of these follicular cells contained fine brown granules within the cytoplasm.

Analysis of minocycline in the lesional skin

The HPLC profile of extracts from pigmented skin and the standard minocycline solution are shown in Fig. 5. On HPLC, the standard minocycline gave a single sharp peak and extracts of

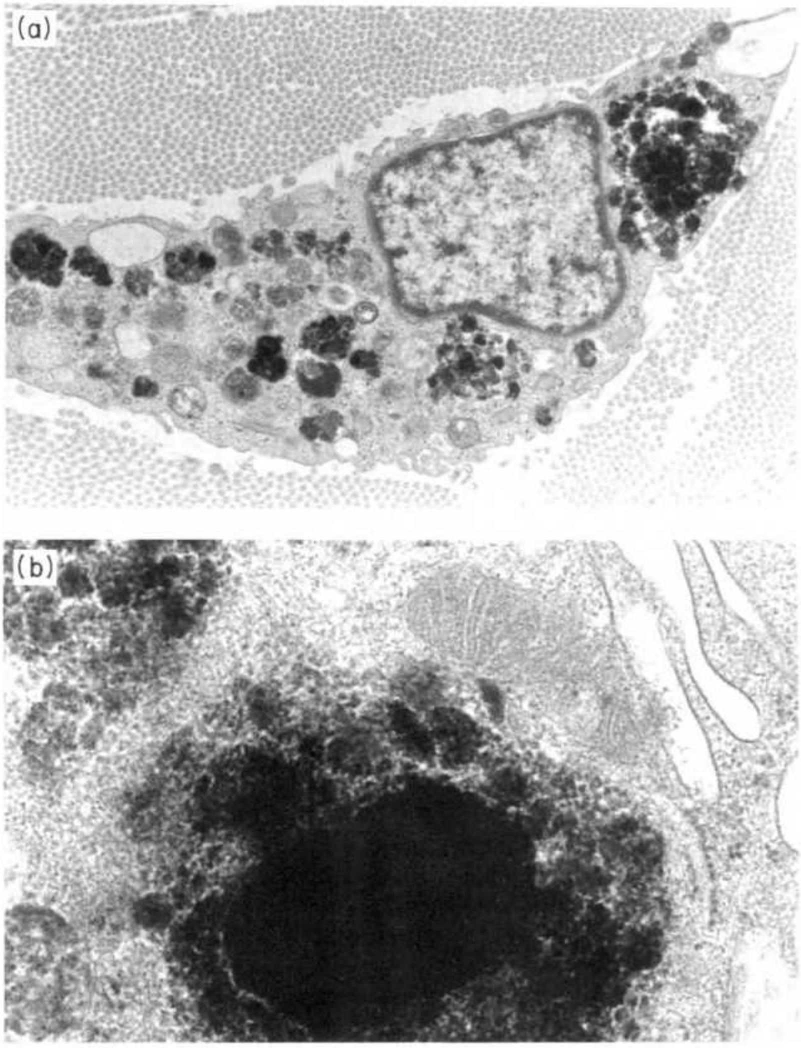

FIGURE 3. (a) Non-membrane-bound electron-dense granules within cytoplasm of a dermal macrophage (× 10,500), (b) Higher magnification of the granules (× 40,500).

the skin sample gave the same peak. Compared with the internal standard the amount of minocycline in the skin tissue was estimated as 13·2 μg/g wet tissue.

DISCUSSION

The presence of minocycline in the pigmented skin was demonstrated by HPLC. This was after the drug had been stopped 2 months before the skin biopsy was taken. The high level of minocycline (13·2 μg/g tissue) suggests an accumulation of this antibiotic in the lesional skin.

Energy-dispersive X-ray microanalysis confirmed the presence of iron in the electron-dense granular material within dermal macrophages. This is similar to results previously reported.[3-5]

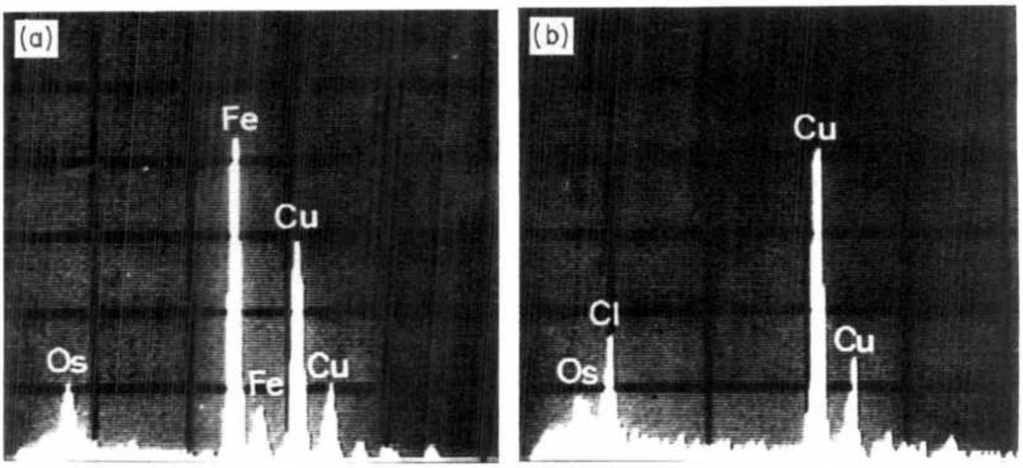

FIGURE 4. X-ray spectrograph of electron-dense deposits (a) shows prominent iron K-alpha (Fe left) and K-beta (Fe right) peaks. These iron peaks are not apparent in the background cytoplasm (b). Fe: iron; Os: osmium; Cl: chloride; Cu: copper.

Haemosiderin or a pigment with haemosiderin-staining characteristics has been observed in skin pigmentation induced by minocycline.[1,2,6] Extravascular leakage of erythrocytes is considered to be the source of haemosiderin and minocycline and its metabolites do not contain iron.[16] Though there were no obvious areas of haemorrhage she had a 10-year history of varicose veins and the pigmentation may have occurred at the sites of previous vascular damage. It is perhaps more likely that the iron containing particles are not haemosiderin but complexes of

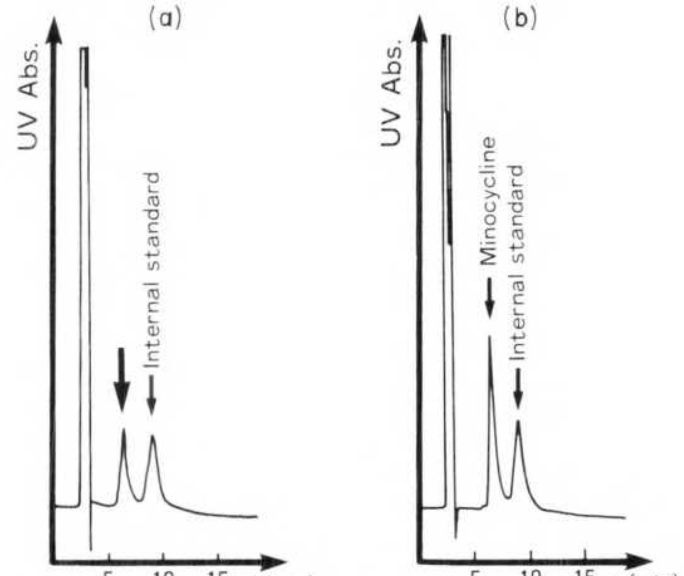

FIGURE 5. HPLC analysis of skin extracts (a) and standard minocycline solution (b). The peak detected in the skin extracts (a) was at the same position as that for minocycline in (b).

minocycline with iron and other substances. In one study it was found that the particles in the dermal macrophages did not stain for iron and the pigment showed the characteristics of melanin.[7] It was suggested that minocycline might stimulate melanin pigmentation which was accentuated by exposure to sunlight. In our case the amount of melanin was increased in the basal layer of the epidermis with some incontinence to the dermis.

Tissue distribution studies using radiolabelled minocycline in animal models showed that higher levels of radioactivity were detected in thyroid, brain, bone and skin than similar studies with other tetracyclines.[16,17] In the present case the follicular epithelial cells of the thyroid also contained fine pigmented granules within the cytoplasm. Although we did not determine the nature of the pigment in the thyroid, it is likely that this pigment is also associated with minocycline treatment. Laboratory animals fed with high doses of oral minocycline develop a pronounced black discoloration of the thyroid gland.[18] Pigmentation associated with minocycline therapy has been seen in surgical and autopsy specimens of thyroid glands.[10–12,14] In all these cases the pigment was localized in the cytoplasm of the follicular epithelial cells of the thyroid gland.

The mechanism by which minocycline produces pigment in the skin is not well understood. Minocycline is a yellow crystalline material, but can change to black.[11,13,19] This could explain the development of cutaneous pigmentation in patients taking minocycline. In many of the reported cases, as well as ours, the minocycline-related skin pigmentation has been predominantly on the legs.[2,3,5,6]

Considering the amount of minocycline prescribed world-wide, the number of reported cases of increased skin pigmentation is few. The cutaneous pigmentation usually resolves spontaneously after stopping this antibiotic but can be persistent.

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Late onset erythropoietic porphyria

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SUMMARY

A 51-year-old Japanese man and his 56-year-old sister of consanguineous parents had skin lesions with areas of dark-brown pigmentation and blisters with minimal trauma on sun-exposed skin which resembled those seen in porphyria cutanea tarda. Their fresh urine was wine-red in colour and fluoresced with ultraviolet light. The peripheral blood contained fluorocytes and porphyrin analysis of the red blood cells, urine and faeces of the patients revealed an increase of the isotype I of uro- and coproporphyrin and normal concentrations of δ-aminolaevulinate and porphobilinogen, suggesting the diagnosis of erythropoietic porphyria. No other members of this family had symptoms or biochemical findings suggestive of porphyria. We consider these two cases to be that of late onset erythropoietic porphyria.

The classification of porphyria is based on the genetic aspects, the types of porphyrin precursors appearing in the blood, urine and faeces, and the enzymatic deficiencies relating to porphyria metabolism.[1] Most cases can be classified as erythropoietic porphyria of Günther, erythropoietic protoporphyria, acute intermittent porphyria, porphyria variegata, porphyria cutanea tarda or hereditary coproporphyria.[1] A few unusual forms of porphyria have been reported and new types of porphyria have recently been proposed, such as hepatoerythropoietic porphyria[2] and erythropoietic coproporphyria.[3] Congenital erythropoietic porphyria is very rare and usually becomes apparent in early infancy, but several cases have been reported in which clinical symptoms started in adult life.[4–7] We report two siblings with skin lesions that resembled porphyria cutanea tarda but with biochemical findings that suggested the diagnosis of erythropoietic porphyria.

CASE REPORTS

Case 1

A 51-year-old Japanese man presented with a 25-year history of photosensitive eruptions on the face and hands. The lesions consisted of blisters which developed following minimal trauma in

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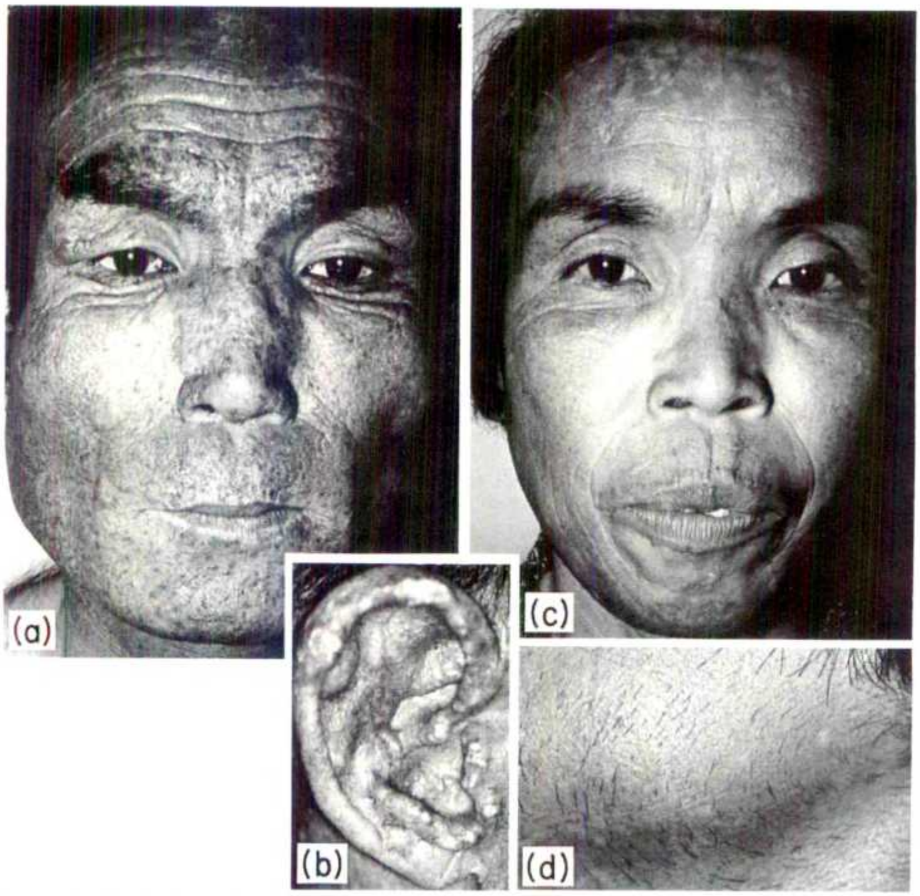

FIGURE 1. (a) Case 1 Numerous tiny pitted scars and hyperpigmented macules are diffusely distributed on the face. Depigmented areas are seen on the forehead. The bulbar conjunctiva has pigmented dots. (b) Case 1 Milia are present on the pinna. (c) Case 2 Diffuse hyperpigmentation with depigmented areas are present on the face and forehead. (d) Case 2 Hypertrichosis on the angle of the jaw.

the light-exposed areas. These were crusted ulcers which healed to leave scars and many milia were also present. Examination revealed numerous confluent dark-brown macules with many tiny scars on the face, forehead and dorsum of both hands. Some pigmented dots were also seen on the bulbar conjunctive (Fig 1a, b). Hypertrichosis was not present. After exposure to the sun the patient felt fatigued and noticed that his urine was dark-red in colour. His parents were first cousins, and one of his six siblings had similar but less severe symptoms (Case 2). His parents and children were healthy and had no symptoms of photosensitivity. He consumed a small amount of alcohol each day but there was no history of exposure to chemicals or drugs that might induce porphyria. There was some hepatomegaly, but neither gallstones nor splenomegaly were detected by CT-scan. Laboratory investigations did not indicate any anaemia (red blood cell count: 479×10^4/cmm, reticulocytes: 3·5%, haemoglobin: 16·0 g/dl) but did reveal mild liver dysfunctions (GOT, 33 iu/l; GPT, 17 iu/l; LDH, 620 iu/l; total bilirubin, 1·2 mg/dl).

The histology of a skin biopsy specimen from the forehead revealed hypermelanosis of the basal cells of the epidermis and marked thickening of the walls of the dermal blood-vessels with periodic acid Schiff positive material. On electron microscopy this was shown to consist of concentric and multilayered basal lamina with amorphous material surrounding the endothelial cells and pericytes. No inflammatory infiltrate was noted in the dermis but a few lymphoid cells were found in relation to the blood-vessels (Fig. 2a, b).

Case 2

A 56-year-old housewife, sister of Case 1, had noted dark-brown pigmentation on her forehead, cheeks and on the dorsa of both hands over the previous 20 years. The pigmentation was diffusely distributed on the forehead and there were irregular shaped hypopigmented areas within the hyperpigmented areas. She also had mild hypertrichosis of the skin on her chin (Figs 1 c, d). She had noted that her urine was wine-coloured when she was tired. During a pregnancy she had blisters on the backs of her hands. She had no hepatosplenomegaly and the laboratory

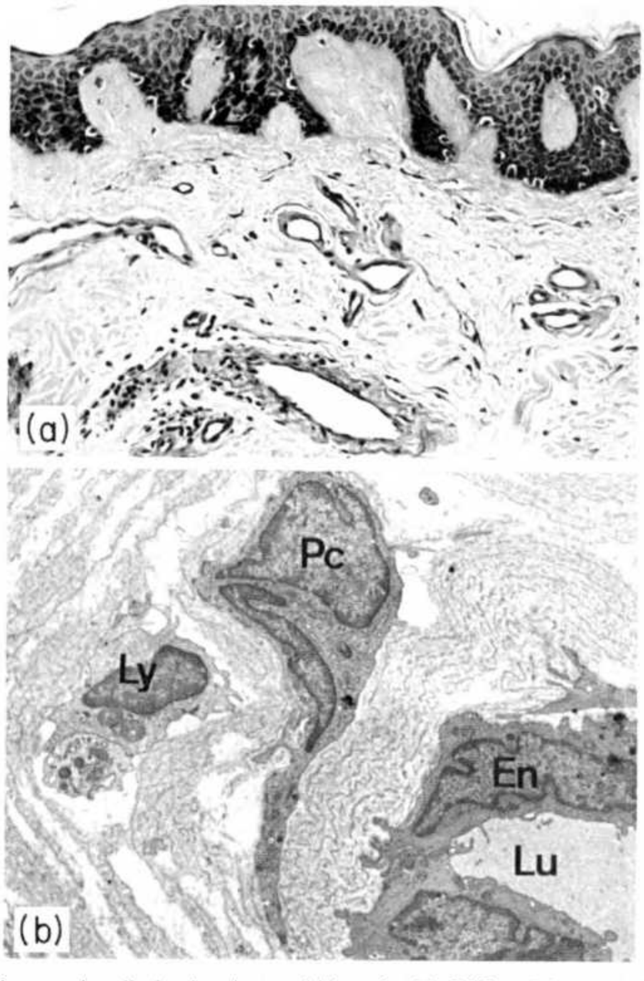

FIGURE 2. (a) The vessel walls in dermis are thickened with PAS-positive material. (b) Electron microscopy of a dermal vessel shows a multilayered basal lamina with amorphous granular material. Lu, capillary lumen; En, endothelial cell; Pc, pericyte; Ly, lymphoid cell.

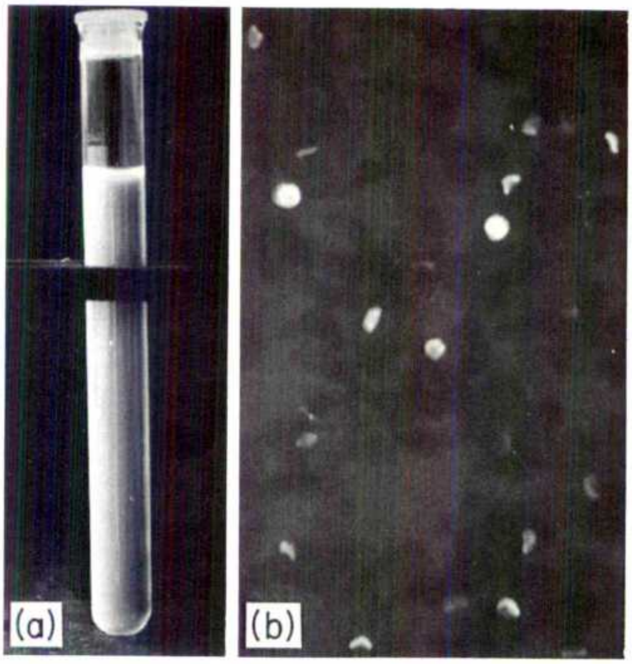

FIGURE 3. (a) The fresh urine of Case 1 showing autofluorescence. (b) Fluorocytes of Case 1.

examinations did not indicate any anaemia (red blood cell count: 455×10^4/cmm, reticulocytes: 1·3%, haemoglobin: 13·5 g/dl) but the LDH level was increased (699 iu/l). The skin biopsy taken from her forehead showed the same changes as in Case 1.

Light irradiation tests

Although their teeth did not show fluorescence (erythrodontia), the patients' urine showed a marked pink fluorescence with illumination under a Wood's lamp (Fig. 3a). Red blood cells in smears from each patient showed autofluorescence (fluorocytes), which soon diminished (Fig. 3b). The younger daughter of Case 1 also showed only a few fluorocytes in a high-power field that diminished too soon to be able to photograph.

Porphyrin and enzyme analysis

The concentrations of uro-and coproporphyrin in the urine and uro-, copro- and protoporphyrin in the peripheral red blood cells and in the faeces were determined in each case by established methods.[8,9] The isotype of coproporphyrin was determined using high-pressure liquid-chromatography.[10] Concentrations of δ-aminolaevulinate and porphobilinogen were determined,[11] and the activity of uroporphyrinogen I synthetase and uroporphyrinogen decarboxylase in the peripheral red blood cells in Case 1 was measured.[10,12] The concentration of the coproporphyrin in the urine of their mother, their siblings and the children of each case was also measured.

Table 1 shows the concentrations of the porphyrin precursors in Cases 1 and 2, and Figure 4 those of urinary coproporphyrin in members of the family. In both patients, uro- and coproporphyrin were detected at a high concentration in the urine, red blood cells and faeces, and the isotype of the coproporphyrin was isomer I. The concentrations of δ-aminolaevulinate

TABLE 1. Level of porphyrin precursors

	Red cell			Urine				Faeces		
	Uro	Copro	Proto	ALA	PBG	Uro	Copro	Uro	Copro	Proto
	(μmol/l packed cell)			(μmol/l)		(nmol/l)		(nmol/g dry weight)		
Case 1	3301	1445	1807	12·5	trace	8140	6223	114	3421	76
Case 2	494·	458	1192	8·5	0	2083	2040	87	855	48
Normal range	0–trace	0–24	490–1200	10–50	0–10	0–25	0–150	0–4	0–65	0–180

and porphobilinogen were within normal ranges. All other members of their family had a urinary coproporphyrin level within the normal range. The enzyme activity of uroporphyrinogen I synthetase was 75 nmol uro formed/ml RBC/h (at 37°C) (normal range: 38–74 nmol/ml RBC, $n = 7$) and uroporphyrinogen decarboxylase was 24·6 nmol copro III formed/ml RBC/h (at 37°C) (normal range: 19·4–24·4 nmol/ml RBC/h, $n = 7$) in Case 1.

DISCUSSION

These two patients were siblings whose parents were first cousins. No other members of their family, including their mother and their children, had disturbed porphyrin metabolism, as detected by urinary coproporphyrin levels that were markedly increased in both of our patients. The abnormality of porphyrin metabolism in this family appears to have been inherited as an autosomal recessive trait, the patients being homozygous for this gene.

The late onset of clinical features suggests porphyria cutanea tarda or variegate porphyria. However, there was no history of the exposure to drugs or chemicals that may induce porphyria cutanea tarda. The patients had no neural or visceral symptoms that are common in variegate porphyria. The mutilations of the fingers, erythrodontia and haemolytic anaemia with an associated splenomegaly which are prominent features of congenital erythropoietic porphyria were not observed in our patients. However, there was marked accumulation of uro- and

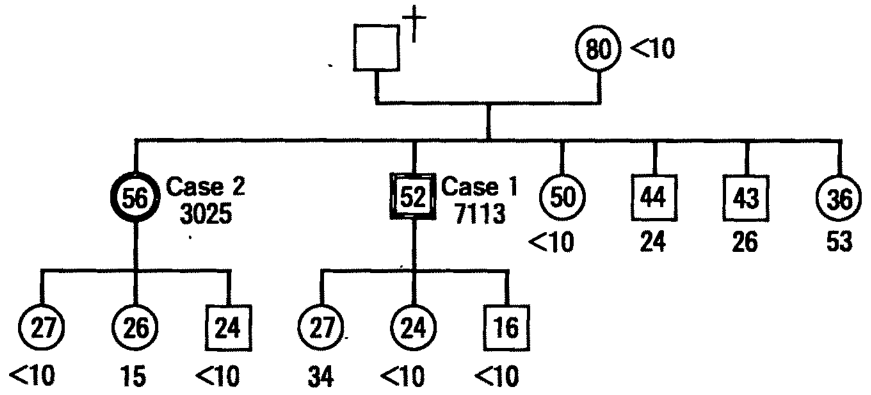

FIGURE 4. Pedigree of the family, showing level of urinary coproporphyrin (nmol/l). Normal range is 0–150 nmol/l. ○, female; □, male. The numbers in symbols are the ages.

TABLE 2. Previous cases of late onset variant of erythropoietic porphyria

							Level of porphyrin precursors									
							Red cell			Urine				Faeces		
Case	Year	Sex	Age of onset (y)	Clinical features	Hb (g/dl)	Reticulocyte (%)	Uro	Copro	Proto	ALA	PBG	Uro	Copro	Uro	Copro	Proto
1[10]	1965	M	54	Pigmentation of sun-exposed areas. Facial hypertrichosis, splenomegaly and red urine	4	16	$\frac{90\cdot3^*}{0}$	$\frac{26\cdot4}{1\cdot5}$ (μg/dl)	$\frac{45\cdot4}{50\cdot0}$	ND†	$\frac{(-)}{2}$ (mg/l)	$\frac{9100}{30}$ (μg/l)	$\frac{3450}{200}$	$\frac{(+)}{(-)}$	$\frac{1562}{36}$ (μg/g dry weight)	$\frac{111}{113}$
2[11]	1975	M	58	Recurrent blistering on exposure to the sun. Facial hypertrichosis. Absence of hepato-splenomegaly, red urine	8·3	10·4	$\frac{2179}{0}$	$\frac{994\cdot5}{31}$ (nmol/l packed cells)	$\frac{178}{890}$	$\frac{38\cdot9}{45\cdot8}$ (μmol/24 hr)	$\frac{10\cdot6}{8\cdot8}$	$\frac{9240}{60}$ (nmol/24 hr)	$\frac{1652}{\text{ND}}$	ND	$\frac{2050}{77}$ (nmol/g dry weight)	$\frac{195}{113\cdot5}$
3[12]	1976	M	50	Vesicular eruption in exposed areas. Lethargy, red urine	9·6	2·6	ND	ND	ND	ND	ND	$\frac{98}{\text{ND}}$ (mg/day)	$\frac{240\cdot8}{\text{ND}}$	ND	$\frac{1292}{\text{ND}}$ (μg/g dry weight)	ND
4[13]	1981	M	36	Recurrent blistering in light exposed areas. Red urine, koilonychia, absence of hypertrichosis, red urine	11·4	7	$\frac{110}{\text{trace}}$	$\frac{1900}{170}$ (nmol/l)	$\frac{5590}{1200}$	$\frac{2}{3}$ (μmol/mmol of creatine)	$\frac{0}{1\cdot5}$	$\frac{1660}{4}$ (nmol/mmol of creatinine)	$\frac{450}{25}$	$\frac{40}{\text{trace}}$	$\frac{3090}{60}$ (nmol/g dry weight)	$\frac{110}{110}$
5[13]	1981	M	23	Recurrent blistering on the face and hands. Scars, koilonychia and red urine	normal	4·4	$\frac{500}{\text{trace}}$	$\frac{1125}{170}$ (nmol/l)	$\frac{4520}{1200}$	$\frac{2\cdot3}{3}$ (μmol/mmol of creatine)	$\frac{0}{1\cdot5}$	$\frac{1660}{4}$ (nmol/mmol of creatine)	$\frac{400}{25}$	$\frac{39}{\text{trace}}$	$\frac{2330}{60}$ (nmol/g dry weight)	$\frac{120}{110}$

$^* \dfrac{\text{level of porphyrin}}{\text{upper limit of control}}$.

† not described.

coproporphyrin in the urine, red blood cells and faeces, with normal concentrations of delta-aminolaevulinate and porphobilinogen suggesting that both cases should be classified as erythropoietic porphyria of Günther.[1] Since the assays of enzyme activity of uroporphyrinogen I synthetase and uroporphyrinogen decarboxylase revealed no deficiencies of these enzymes, and the increased isomer of porphyrin was type I of uro-and coproporphyrin, the enzymatic defect in these cases seems to be that of uroporphyrinogen III cosynthetase, as in erythropoietic porphyria.[13] Although the enzymes were only measured in one of the two cases, it is assumed that both had the same disorder since they are siblings. Unfortunately we were unable to measure uroporphyrinogen cosynthetase.

Although most cases of erythropoietic porphyria are congenital (Günther's disease), several have been reported that have had a late onset of photosensitivity similar to that seen in porphyria cutanea tarda and with features on porphyrin analysis that suggest erythropoietic porphyria.[4–7] Table 2 summarizes the clinical features and porphyrin levels in these cases. In one case the estimation of the uroporphyrin concentration in the red blood cells of the members of the family suggested heterozygosity.[5] However, Deybach *et al.*[7] suggested homozygosity for their cases, judging from the levels of uroporphyrinogen III cosynthetase activity in the patients' families, and suspected that the metabolic abnormality was a primary defect of the cosynthetase activity. It appears that our patients were homozygous, and their children who would be heterozygous were found to have normal levels of urinary coproporphyrin. The 27-year-old daughter of Case 1 showed only a few fluorocytes in her blood.

Our patients had milder haematologic abnormalities than previous cases. It is at present unclear whether the enzymatic defect is the same in all cases of late onset of erythropoietic porphyria. However, the late onset of disease in these patients differentiates it not only from the common type, but also from the milder form of congential erythropoietic porphyria.[14] As has been stated,[7] it is clear that there are two diseases: a congenital and a late onset type. Analysis of the gene arrangement, especially studies of restriction enzyme related length polymorphism[15] in the families of both types, may help to clarify the differences in enzymatic deficiencies between the two types.

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Acrodermatitis chronica atrophicans with coexisting morphoea

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SUMMARY

We report a case of acrodermatitis chronica atrophicans with coexisting morphoea in a British native.

Acrodermatitis chronica atrophicans (ACA) is a chronic progressive dermatosis which most commonly affects the skin of the extremities. Recently, evidence has accumulated to suggest that the tick-borne spirochaete *Borrelia burgdorferi*, the agent responsible for Lyme disease[1] is also responsible for this condition.[2–4] We describe what we believe to be the first report of ACA occurring in a British native since the advent of serological diagnosis confirmation. The coexistence of morphoea, which has been claimed by some to have a spirochaetal aetiology,[5,6] developing shortly after the onset of ACA in our case adds further interest.

CASE REPORT

The female patient, now aged 64 years, developed an enlarging erythematous plaque on the dorsal aspect of her left hand over the course of 2 months in the autumn of 1985. The lesion was neither itchy nor tender and was unassociated with any systemic symptoms. Gradually the lesion became violaceous and atrophic, and close inspection revealed the presence of superficial fine telangiectasia (Figs 1 and 2). Although the area was static in size, the intensity of the violaceous colour fluctuated, always being more marked in cold weather. Seven months after the onset of the hand lesion she developed well circumscribed indurated violaceous plaques on the trunk and back which rapidly became shiny, hypopigmented and sclerotic, with the appearance characteristic of established morphoea (Fig. 3). She had no recollection of any insect or tick bites, and denied having arthralgia, neurological or cardiovascular symptoms.

In the preceding 8 years she had visited continental Europe on several occasions, though mostly her visits were weekend trips to large cities (Paris, Brussels, Amsterdam, Koblenz and Luxemburg). In early 1982, she had spent 4 days in rural Normandy. On several occasions,

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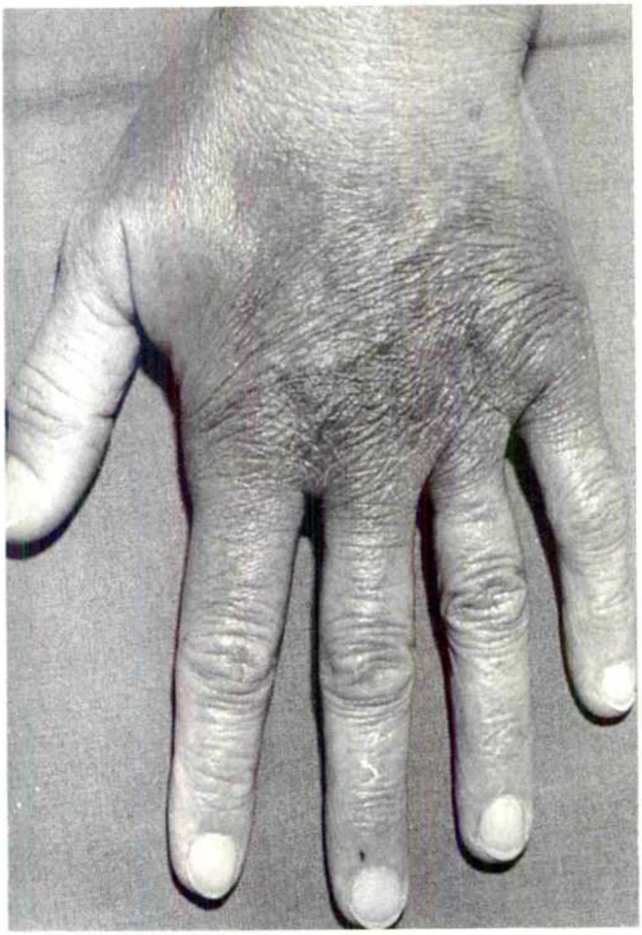

FIGURE 1. The atrophic and violaceous plaque of ACA on the dorsal aspect of the hand.

holidays had been spent away from her south London suburban home at a cottage situated in the heart of the Hampshire New Forest, an area known to be infested by *Ixodes ricinus* ticks infected with the Lyme spirochaete, *B. burgdorferi*.[7]

For 10 years she had suffered from classical necrobiosis lipoidica on both shins. A glucose tolerance test was normal. Investigations revealed a strongly positive indirect immunofluorescent antibody test to *B. burgdorferi* with an IgG titre of 1 in 1024, and an IgM titre at 1 in 256. An IgG ELISA test for *B. burgdorferi* was positive at greater than 100 units. Her latex rheumatoid factor was strongly positive with a rheumatoid factor haemaglutinin titre of 1 in 1280. Results of routine haematology and biochemistry tests were normal.

Histology of a skin biopsy taken from the ACA lesion on the dorsum of the hand showed mild orthokeratotic hyperkeratosis with marked atrophy of the stratum spinosum and loss of the rete ridges (Fig. 4). In the papillary dermis dilated vessels were prominent (Fig. 5). Scattered throughout the papillary and reticular dermis was a marked mixed chronic inflammatory infiltrate which in areas appeared layered between the collagen bundles. There was a predominance of plasma cells in the infiltrate, though in some areas histiocytes and giant cells

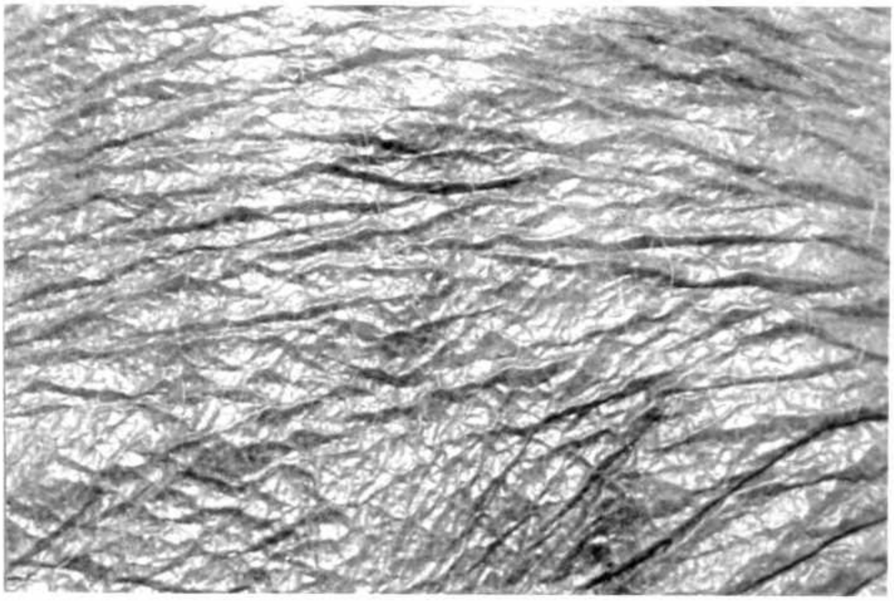

FIGURE 2. Close up of the ACA lesion showing the atrophy, fine wrinkling and telangiectasia.

were conspicuous. Skin biopsy of the lesions on the back showed prominent thickening, homogenization and hyalinization of the dermal collagen. In addition there was a considerable inflammatory infiltrate composed of lymphocytes and plasma cells. These findings supported the diagnosis of inflammatory morphoea.

Skin culture and direct immunofluorescence for *B. burgdorferi* were attempted from a biopsy but the organism was neither identified nor cultured.

Once the diagnosis of ACA was established, a 2 week course of oral tetracycline was started at a dose of 2 g a day. The hand lesion has become much less inflamed though the morphoea remains unchanged. The IgG *B. burgdorferi* immunofluorescence titre, IgG ELISA result and rheumatoid factor were unchanged but the IgM immunofluorescence test is now negative.

DISCUSSION

ACA is an uncommon progressive atrophic dermatosis first described by Buchwald[8] in 1883 which most commonly affects the skin of the extremities. Pick[9] later recognized that an inflammatory phase frequently preceded the atrophy by some years. The disease has been of considerable interest recently because of the establishment of an aetiological role for the tick-borne spirochaete *B. burgdorferi*. Spirochaetal structures have been identified on skin biopsy from lesional ACA skin using light microscopy and the Warthin–Starry silver stain technique.[10] More recently spirochaetes have been cultured from ACA affected skin, albeit from a small proportion of patients.[11] High antibody titres to the spirochaete *B. burgdorferi*, the organism that is now known to be responsible for the multisystem Lyme disease and its cutaneous hallmark, erythema chronicum migrans, have been found in all patients with ACA.[3] In addition, if skin affected by ACA is transplanted to normal skin of another individual, then ACA will

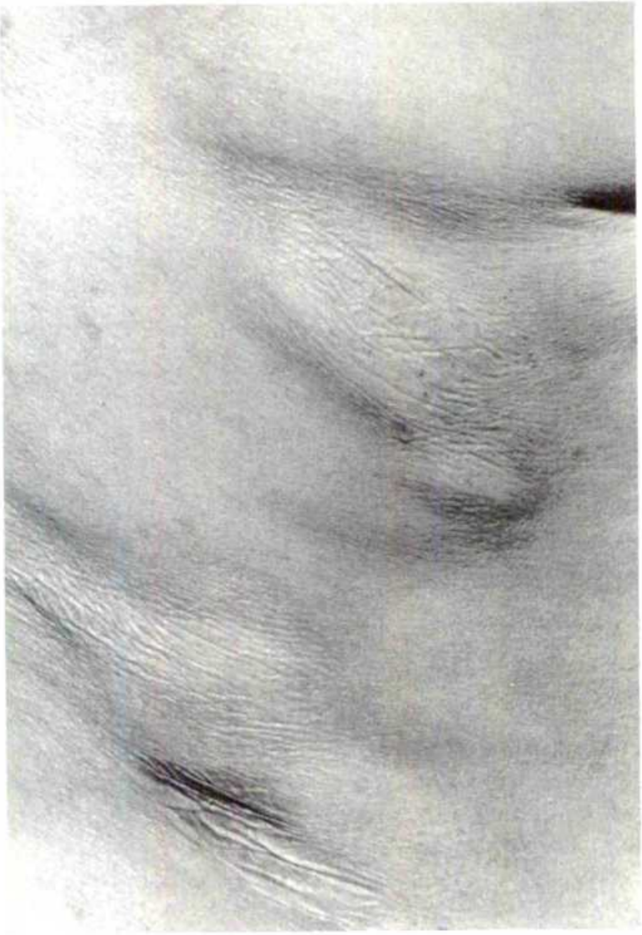

FIGURE 3. A sclerotic plaque of inflammatory morphoea on the back.

develop at the site of transplantation.[3] Lastly, it has been known for many years that penicillin may effect resolution of the lesions of ACA.[12]

The Borrelia spirochaete responsible for ACA has been identified in two species of the hard tick genus *Ixodes*. Many patients with ACA recall having been bitten by ticks and there are a few cases of ACA actually occurring at the site of a tick bite. Other epidemiological evidence supports the role of *Ixodes* ticks acting as vectors for the dissemination of borreliosis.

The clinical and histological features of a series of 32 untreated patients with ACA have been recently reviewed.[3] Lesions are usually solitary and situated acrally, though a small number of patients have multiple lesions which spread onto the trunk and chest. In addition some patients have lichen sclerosus et atrophicus-like lesions in both genital and extragenital sites. Six patients had a history of previous erythema chronicum migrans on the extremity where ACA developed between 1 and 8 years later. Six patients also subsequently developed neurological symptoms which consisted of trigeminal neuralgia, sensineural deafness, facial palsy and vertigo. A much higher proportion of patients developed a peripheral neuropathy. Such neurological deficit is well recognized in Lyme disease, and appropriate antibiotic therapy may prevent such sequelae,

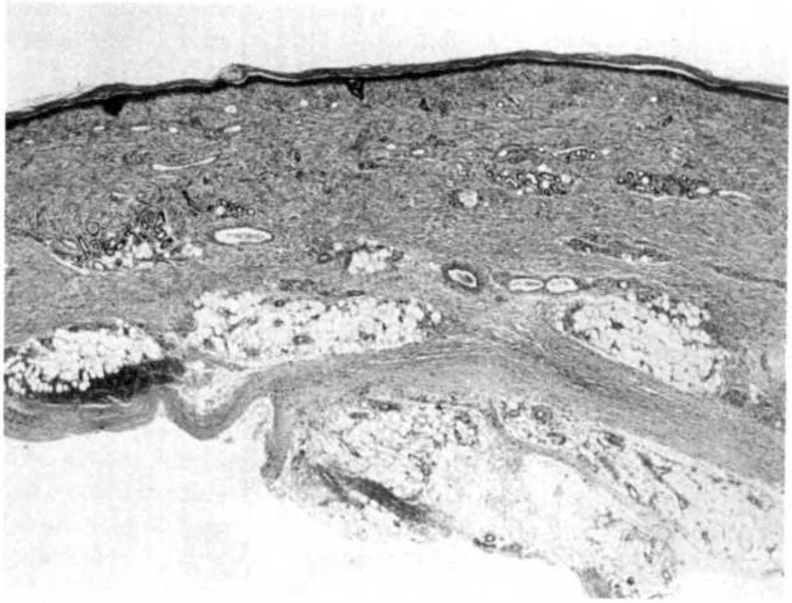

FIGURE 4. Biopsy from the ACA lesion on the hand. Dermal and epidermal atrophy is apparent. (H & E × 28·8)

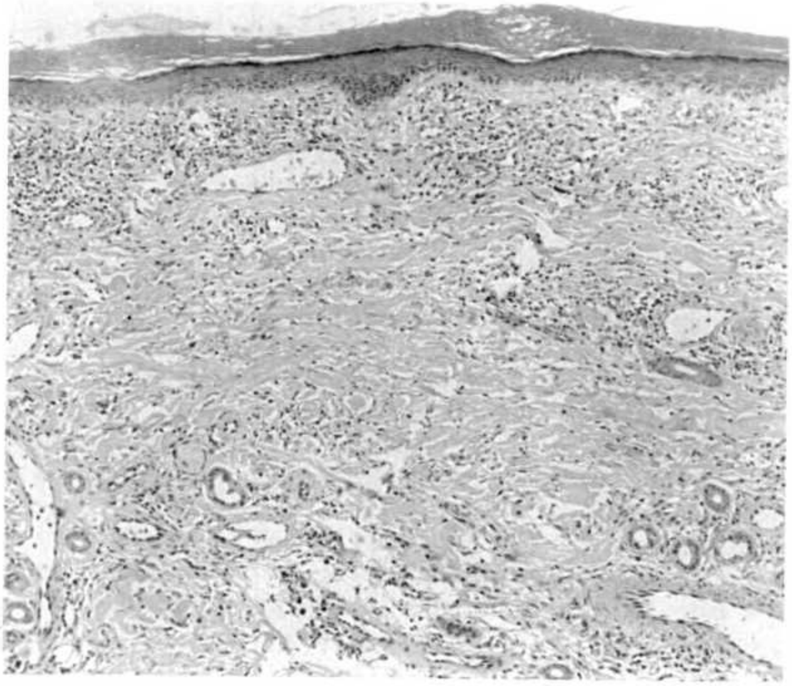

FIGURE 5. Prominant telangiectatic vessels and a mixed chronic inflammatory infiltrate is shown in the dermis of the ACA lesion (H & E × 84)

indicating the importance of the recognition of ACA. Six patients had developed deformities of the feet and hands underlying the ACA lesions. Other authors have commented on the frequency of cardiac symptoms that occur in untreated ACA.[4]

The patient reported above had a typical lesion of established ACA. The histopathology of this condition may vary but the largest review published since confirmation of the diagnosis of ACA by serology has stressed a number of points that our case demonstrates.[3] In most cases there is a lymphocytic infiltrate with a rich admixture of plasma cells. Epidermal atrophy and basal cell liquifactive degeneration may be seen with prominent papillary dermal telangiectasia. The additional histiocytic and giant cell response seen in our case appears to be unusual. Large, as yet unexplained, dermal vacuoles seen in some cases of ACA were not seen in our case.

The subsequent appearance of clinically and histologically typical inflammatory morphoea after the onset of ACA is of interest, though not unique.[3,13] Spirochaetes with a morphology similar to *B. burgdorferi* have recently been cultured from two patients with morphoea, both of whom were serum antibody negative by immunofluorescence and ELISA[6]. Aberer *et al.*, prompted by the clinical and histological similarities between ACA and morphoea, found five out of 10 patients with morphoea to have significant antibodies measured by ELISA to *Borrelia burgdorferi;* three of their 5 antibody negative patients had received high doses of penicillin.[5] Muhlemann *et al.*[14] modified the immunofluorescent *B. burgdorferi* antibody tests by the addition of FTA-ABS to the test system. This produced at least a 2-fold reduction in the antibody titre level in those patients with morphoea with positive IgG class antibodies to *B. burgdorferi* compared to when saline alone was used as the test diluent. There was no change in the antibody titre with FTA-ABS addition in those patients with Lyme disease or erythema chronicum migrans. Thus, the specificity of the test seems to be increased without a loss of sensitivity, and they were then unable to confirm the serological link with morphoea. However, the mounting number of cases of ACA associated with morphoea appears more than coincidental, suggesting that a small proportion of cases of morphoea may result from Borrelia infection.

The association of ACA with classical necrobiosis lipoidica appears to be unique and is probably coincidental. We have been unable to detect positive *B. burgdorferi* antibodies in the serum of 10 other patients with classical necrobiosis lipoidica.

ACA is not uncommon in continental Europe, but is extremely rare in the U.S.A., and to date has not been reported from the U.K. The reason for this is poorly understood, as in the U.S.A., other manifestations of this tick-borne borreliosis (erythema chronicum migrans and Lyme disease) are frequently reported. It is of interest that the incidence of arthritis and neurological involvement varies in series of patients with Lyme disease reported from the U.S.A. and continental Europe. Variations in the antigenic components of the *B. burgdorferi* outer surface protein (OspA) between North American (OspA homogeneous) and European (OspA heterogeneous) isolates have now been documented,[15] and this may account for the difference in Lyme disease spectrum. Whether only the heterogeneous OspA phenotype will produce ACA is as yet unanswered.

To our knowledge, this is the first case of ACA to be reported from the U.K. since serological confirmation has been available. A few cases were seen after the Second World War in London in servicemen returning from the Continent and one other case has been presented to the Dermatology Section of the Royal Society of Medicine (personal communication Dr J.Pegum). We believe that our patient probably acquired her infection in England, despite frequent trips to the continent. Tick bites are usually acquired in rural environments, and on several occasions in the summer before the onset of the ACA she had walked in the New Forest of Hampshire. All of the continental visits had been only to large cities, except for one, when 3 years before the onset of the ACA she had holidayed in rural Normandy.

ACKNOWLEDGMENT

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Eosinophilic pustular dermatosis: an early skin marker of infection with human immunodeficiency virus?

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SUMMARY

Two cases of eosinophilic pustular dermatosis are reported and both were associated with infection by human immunodeficiency virus (HIV-1). We suggest that eosinophilic pustular dermatosis may occur as an early sign of infection with HIV.

Eosinophilic pustular dermatosis is a rare skin condition, first described in 1965 by Ise and Ofuji and seen mainly in Japanese patients.[1,2] One case has been reported from Scandinavia,[3] but to our knowledge this disease has not previously been seen in Denmark. We report two cases that were both associated with infection by human immunodeficiency virus (HIV-1). Both cases caused diagnostic problems.

CASE REPORTS

Case 1

A 70-year-old male presented with a history of several months of very itchy papules that appeared to be follicular and were located on the face, neck and upper back (Fig. 1). Treatment with topical steroid preparations were of no benefit, but 100 mg dapsone daily produced a significant improvement. Oral tetracycline in a dosage of 500 mg daily for a period of 2 months controlled the eruption.

Results of routine laboratory investigations were normal, except for a blood eosinophilia of 11%. A skin biopsy showed a lymphocytic infiltrate around the dermal blood-vessels with some extravasation of red cells. There were also many eosinophils in the dermal infiltrate. No micro-organisms were observed.

The patient was later admitted to hospital with general debility, multiple infections and marked weight loss of 24 kg. Miliary tuberculosis was suspected, but the HIV-1 antigen test was

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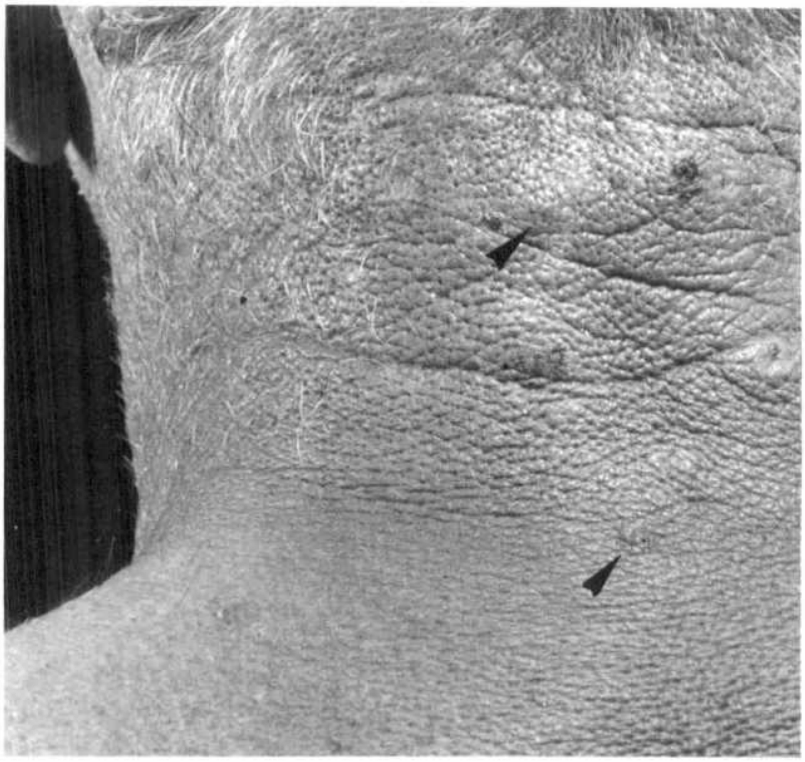

FIGURE 1. Oedematous, scratched, follicular papules located on the back of the neck (arrows: typical lesions).

positive both with the ELISA and Western blot techniques. Soon afterwards he died of a *Pneumocystis carinii* pneumonia.

The patient was heterosexual, but had visited prostitutes in Greece and had spent time in Africa from 1949 to 1962.

Case 2

A 37-year-old male, HIV-1 antigen positive for 2·5 years had mild generalized lymphadenopathy, but was otherwise in good health. He had no loss of weight and no serious infections. For several months he had suffered recurrent, itchy skin lesions that were located on the face, neck, trunk and the upper part of the arms. The lesions were 5–10 mm in diameter, urticarial or papular, often follicular and appeared as a folliculitis. The lesions would occur in crops and resolve spontaneously over the course of 5–7 days, when new lesions might then appear. The skin symptoms resolved following 3 days of treatment with 100 mg dapsone a day.

Bacterial and fungal cultures were negative. Results of routine laboratory investigations were normal, except for a blood eosinophilia of 9%. The histology of the skin biopsy showed a folliculitis with a dense infiltrate that consisted mostly of eosinophils (Figs 2 and 3). There was a sparse perivascular infiltrate with lymphocytes and some eosinophils were seen in the upper dermis. Slight spongiosis was present in the basal layer of the epidermis and mild oedema in the papillary dermis. No micro-organisms were detected.

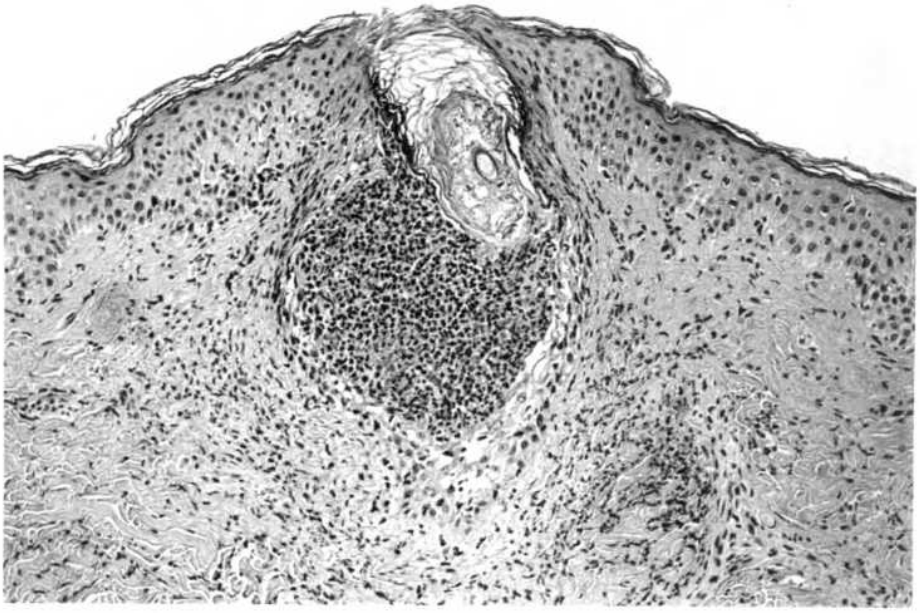

FIGURE 2. Eosinophilic folliculitis. Microabscess in the infundibulum, spongiosis of the outer root sheet and oedema in the surrounding connective tissue (H & E × 94).

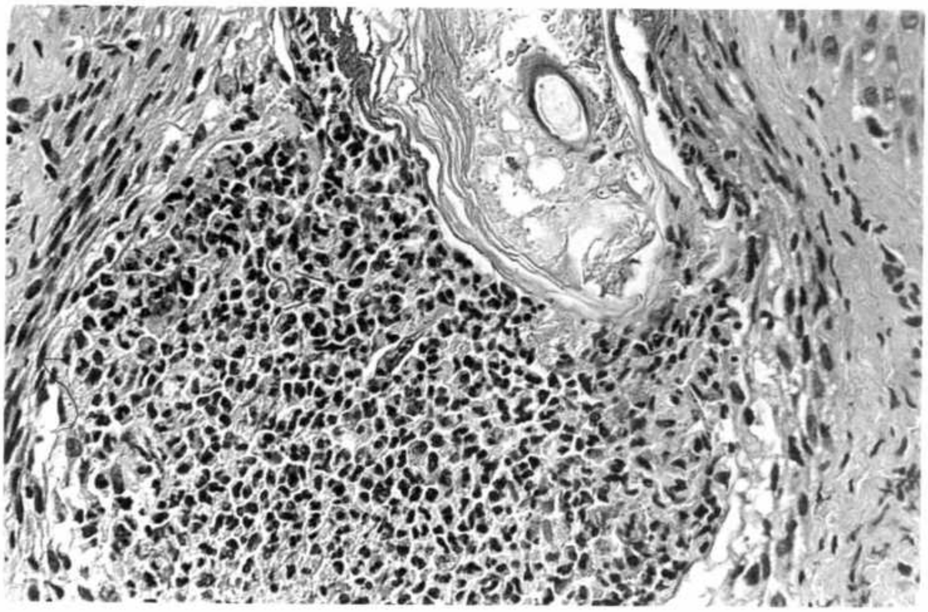

FIGURE 3. Infundibular eosinophilic microabscess in detail. More than half of the inflammatory cells are typical binucleated eosinophils, some with degranulation (H & E × 187·5).

DISCUSSION

These two cases showed identical clinical features with exacerbations and remissions of itchy, oedematous papules and follicular pustules located on the face, neck and upper trunk. Both patients were in good general health at the onset of their skin symptoms, although one was known to be infected with HIV-1, and the other was later diagnosed as having an HIV-1 infection. Both cases responded to oral dapsone.

Soeprono and Schinella[2] have recently reported three cases of eosinophilic pustular folliculitis associated with HIV-1 infection, and suggested that the skin condition represented an abnormal reaction to skin saprophytes or dermatophytes. We did not diagnose our two patients as having eosinophilic pustular dermatosis until the first died from an HIV-1 related infection. We then recognized the similarities between our cases and those reported by Soeprono and Schinella. Both our patients had a blood eosinophilia and there were many eosinophils in the skin biopsies. Unlike the cases reported,[2] our patients had no lesions on the palms or soles and there were no polycyclic lesions with central healing and with papulovesicular or pustular margins. Nor were there other signs of HIV-1 infection.

Recently, Muhlemann *et al.*[4] reported that a chronic acneiform folliculitis on the face, chest and buttocks might be an early warning sign of HIV-1 infection. This occurred in about half their patients who had persistent generalized lymphadenopathy, but no overt acquired immunodeficiency syndrome (AIDS). These lesions were apparently very similar to those seen in our patients. We suggest that the clinical features seen in our patients may indicate an underlying asymptomatic HIV infection. Whether the occurrence of eosinophilic pustular dermatosis might have prognostic implications for the HIV infection is, as yet, not known.

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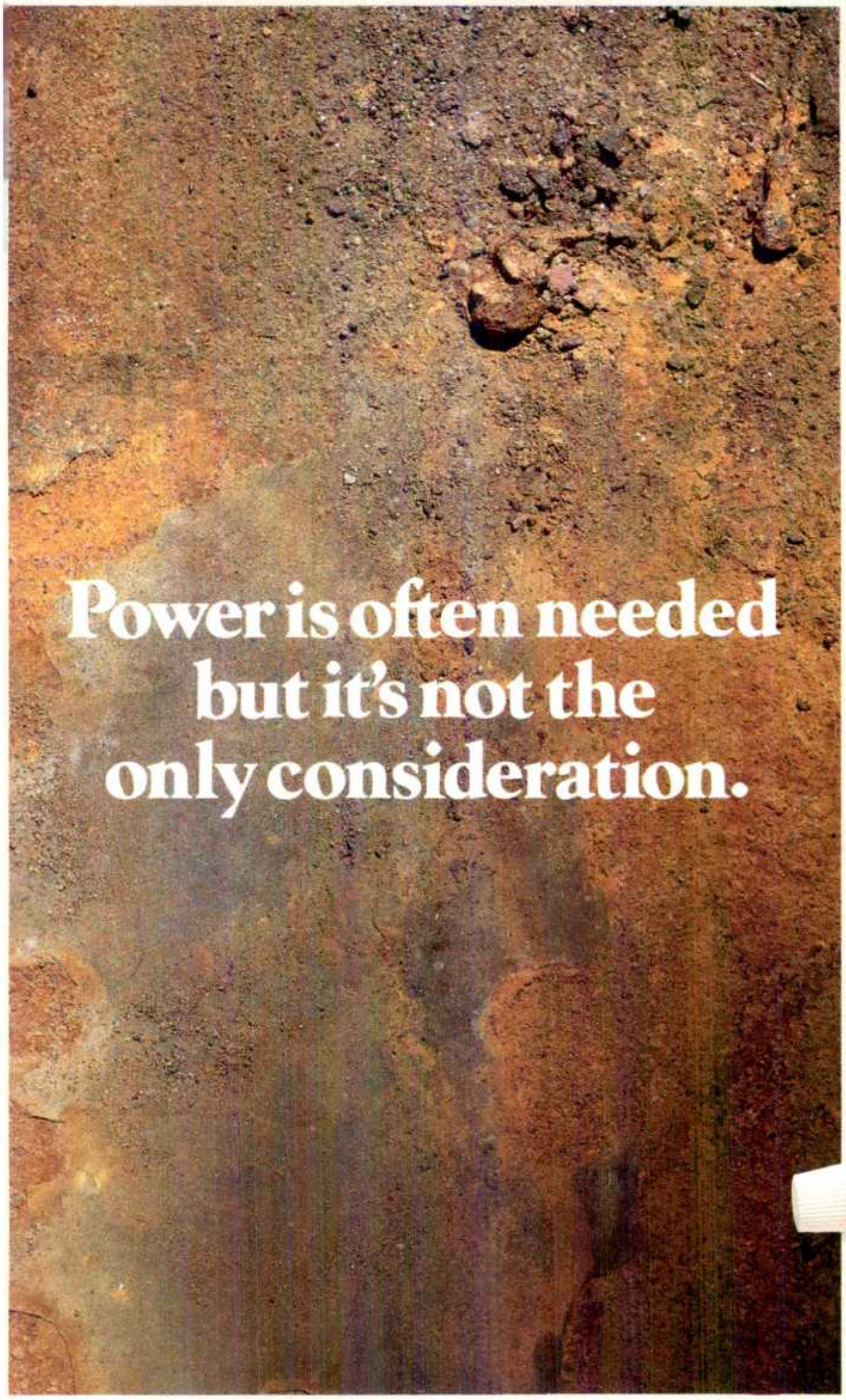

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Epidermal MHC class II antigen expression in systemic sclerosis

SIR, The capacity of the epidermis to synthesise the cytokines interleukin-1, transforming growth factor beta and fibroblast growth factor might be relevant to the pathogenesis of the increased collagen synthesis and the dermal inflammatory cell infiltrate in systemic sclerosis. Moreover, since gamma-interferon may be released from immunocompetent cells in this infiltrate we wished to examine the major histocompatibility complex (MHC) class II lymphocyte antigen expression (HLA-DR, -DP and -DQ) and Langerhans cell density in the skin of patients with systemic sclerosis.

Skin biopsies were taken from sun-exposed clinically involved digital skin and sun-protected clinically uninvolved skin on the medial aspect of the upper arms of 13 patients with systemic sclerosis (SS) (12 females, one male; mean age 50 years, age range 19–67). Eleven of these patients had diffuse cutaneous involvement and two patients had limited acral cutaneous sclerosis with calcinosis. The results were compared with control biopsies taken from similar sites from four age- and sex-matched healthy volunteers (HV) (four females; mean age 49, age range 37–63) and four patients with primary Raynaud's phenomenon (RP) (four females; mean age 49, age range 45–55). Using standard indirect immunoperoxidase techniques, sections were stained with the following monoclonal antibodies (Table 1). The number of positively stained nuclei of Langerhans cells within the epidermis were counted at × 400 and with the aid of an ocular scale were quantified as the number per linear mm of surface epidermis.

The monoclonal antibody DA6·231 produced intercellular staining of the keratinocytes with a chicken-wire pattern throughout the epidermis in all the controls and patients with systemic sclerosis, but staining was more intense in the latter specimens. Langerhans cell bodies and their dendritic processes and cells lining the acrosyringium stained strongly with all the antibodies used although more intense staining was noted with DA6·231. Keratinocyte staining was not seen with Leu 10 and only a few cells of the external root sheath stained with B 7/21. Both DA6·164 and DA6·147 produced a patchy chicken-wire staining of the epidermis in five of the involved and clinically uninvolved biopsies from the patients with systemic sclerosis but in none of the control biopsies. When present this was often localized to an area above the papillary blood-vessels. Staining of keratinocyte membranes adjacent to cells of the acrosyringium, external root sheath and Langerhans cells also was seen with DA6·164 and DA6·147 in all the biopsy specimens. All the monoclonal antibodies except Na 1/34 stained the perivascular mononuclear cellular infiltrate and the intravascular endothelium of control and patient biopsy specimens. Na 1/34 stained only a small percentage of the perivascular infiltrate and a few dendritic cells within the dermis. The mean number and SEM of Langerhans cells per linear mm of surface epidermis in the sun-exposed involved digital and sun-protected uninvolved upper arm skin were 6·9 ± 1·87 and 13·1 ± 1·2 (HV), 6·1 ± 1·02 and 13·8 ± 1·61 (RP) and 5·4 ± 1·64 and 18·2 ± 1·81 (SS) respectively. Comparison of the differences in numbers of Langerhans cells between corresponding sites was not significant.

TABLE 1.

Monoclonal antibody	Dilution	Source	Specificity
Leu 10	1:50	Becton Dickinson	HLA-DQ
B 7/21	Neat	I. Trowbridge	HLA:DP
DA6·147	Neat	K. Guy	HLA-DR (α chain) and HLA-DQ
DA6·164	Neat	K. Guy	HLA-DR (β chain)
DA6·231	Neat	K. Guy	HLA-DP, HLA-DQ, HLA-DR
Na/134	1:1000	A. McMichael	CD1

This study failed to detect any absolute differences in class II antigen expression and density of Langerhans cells between the skin of patients with systemic sclerosis, patients with primary Raynaud's phenomenon and healthy volunteers. The lack of difference in these parameters in the skin of patients with systemic sclerosis makes it unlikely that keratinocytes contribute to the pathogenesis of the dermal inflammatory and fibrotic processes.

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Is the atypical neutrophilic dermatosis with subcorneal IgA deposits a variant of pemphigus foliaceus?

SIR, In 1982 Wallach and co-workers described an apparently new blistering disease under the term 'subcorneal pustular dermatosis and monoclonal IgA'.[1] Since then, similar cases have been reported.[2–8] In 1987 Wallach *et al.* coined the term atypical neutrophilic dermatosis with subcorneal IgA deposits for this new entity.[7]

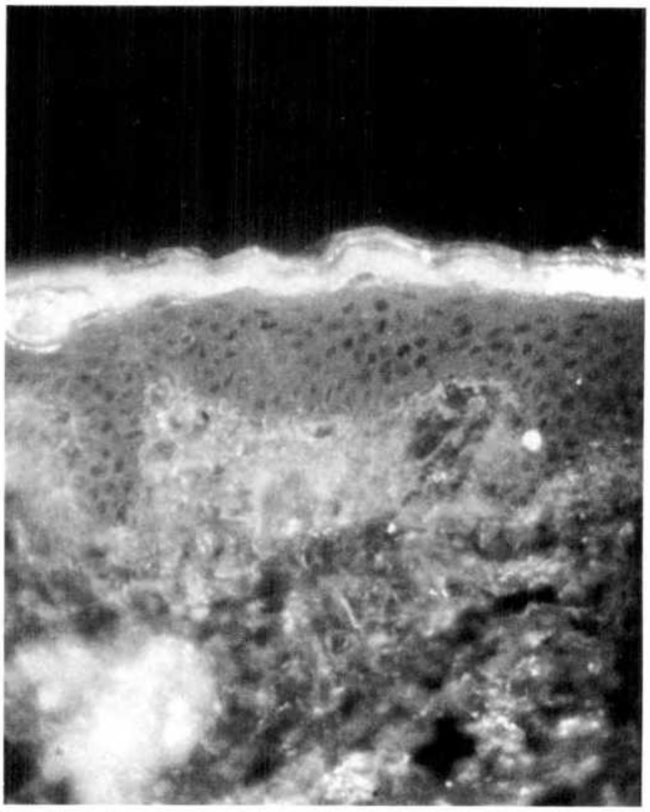

FIGURE 1. IgA deposits within the horny layer and in some areas between cells of the uppermost spinous layer detected by direct immunofluorescence microscopy.

We report an immuno-electron microscopic study of a 76-year-old female patient with atypical neutrophilic dermatosis and subcorneal IgA deposits (ANDSA). Almost symmetrical nummular, erythematous lesions with vesicles were present on the trunk. The vesicle formation was transient, leading to erosions which were covered by crusts. The lesions tended to form circinate and annular patterns, and healed within weeks leaving behind superficial, slightly atrophic scars or hyperpigmented macules.[9]

On direct immunofluorescence microscopy of the lesional skin, IgA deposits were located within the horny layer and in some areas between cells of the uppermost spinous layer (Fig. 1). A similar pattern was seen with indirect immunofluorescence, when normal human skin was incubated with the patient's serum and goat anti-human IgA/FITC.

For immuno-electron microscopy of perilesional skin, the pre-embedding multi-step peroxidase–antiperoxidase technique was used.[10] Deposits of IgA immediately underneath the horny layer and within the intercellular spaces were found (Fig. 2). The IgA precipitates were closely associated with the cell surface membrane and especially within dilated intercellular spaces, in which the number of desmosomes appeared to be decreased. In some intercellular spaces, which appeared only slightly widened, granular IgA deposits could be found in close relationship to desmosomes (Fig. 3). Ultrastructural signs of parakeratosis were also present, such as lipid droplets in the upper spinous and the horny layer (Fig. 2), and with an absence of the stratum granulosum. Immunoprecipitates could not be detected by the pre-embedding technique in the horny layer, which may have been due to technical reasons.

Our immuno-electron microscopic findings provide evidence that IgA antibodies in ANDSA bind to the surface of keratinocytes in the upper spinous layer and may accumulate in the region of the desmosomes. This pattern resembles the ultrastructural localization of IgG precipitates in pemphigus vulgaris.[11,12] To our knowledge, immuno-electron microscopic investigations of pemphigus foliaceus have not been published.[13] However, a loss of desmosomes and widening of intercellular space within the upper spinous

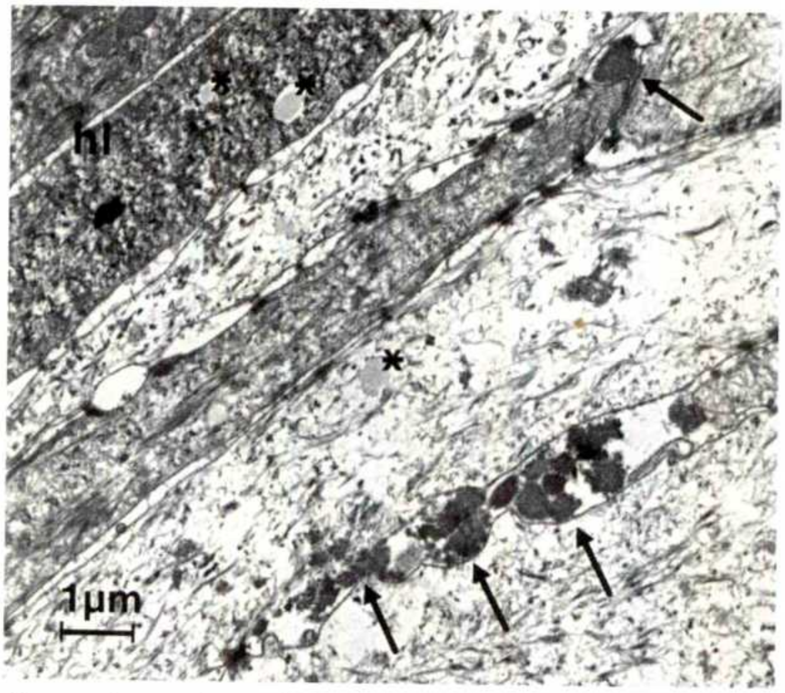

FIGURE 2. Immuno-electron microscopic deposits of IgA (→) immediately underneath the horny layer (hl) within the intercellular spaces in close association with the cell surface. Note wide intercellular space with loss of desmosomes, absence of stratum granulosum, and lipid droplets (*).

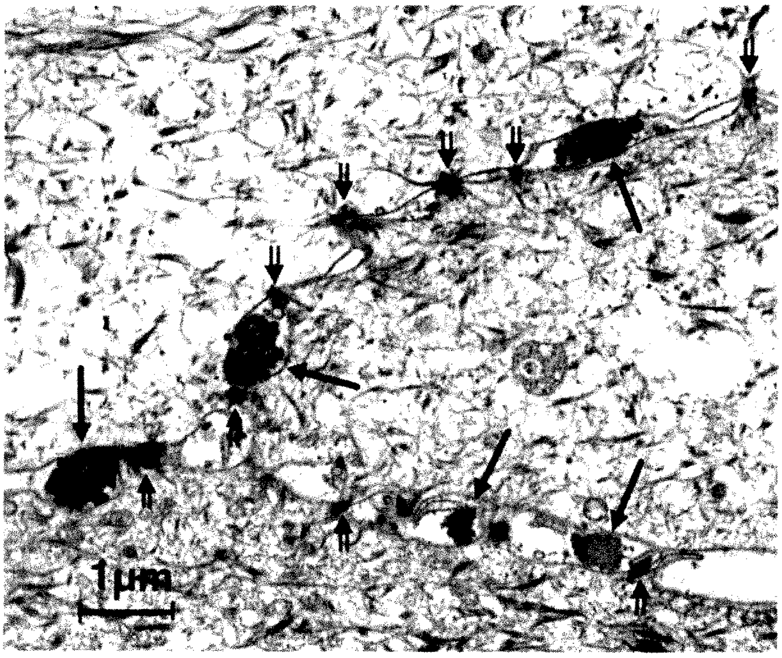

FIGURE 3. IgA deposits (→) accumulating in desmosomal areas. Note the only slightly widened intercellular space and the presence of desmosomes (⇒).

layers as seen in our patient was detected by electron microscopy in pemphigus foliaceus[14] and within the Malpighian layer in pemphigus vulgaris.[15] We suggest that ANDSA may be related to, or may even represent, a IgA variant of pemphigus foliaceus.

Further evidence for this hypothesis is provided by routine histology, where acantholysis within the upper spinous layer was seen in an initial lesion of our patient. Also, indirect immunofluorescence revealed the presence of pemphigus-like circulating IgA antibodies directed against the upper spinous layer, as in other reported cases of ANDSA.[1,5-8]

In pemphigus foliaceus, desmosomes within the spinous layer have been shown to carry the epitope for IgA antibodies.[16,17] The relationship between pemphigus foliaceus and ANDSA awaits confirmation by characterization of the antigen specificity of circulating IgA antibodies.

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Role of *Propionibacterium acnes* in cancer risk

SIR, The report of Dr Sheehan-Dare *et al.* on the role of *Propionibacterium acnes* in cancer risk indicates that acne during puberty may confer some protection against malignant disease.[1] The authors found a reduced incidence of acne of patients with lymphomas and leukaemias. We have done a similar study showing no such reduced susceptibility to malignancy.[2] We compared the acne histories of 86 cancer patients and 86 controls matched for sex and age (± 1 year, range 25–50 years). Several malignancies were included; the predominant types were mammary, gastrointestinal, urogenital and CNS tumours. Cancer patients had suffered from acne more often than controls (36% vs. 29%). Moreover, cancer patients scored higher with regard to the severity, the extent and the persistence of the acne. None of the differences reached statistical significance.

We conclude that acne during puberty and adolescence in all probability does not protect against cancer. If anything, our results suggest that a history of extensive or persistent acne is more common in cancer patients than in controls. Our data contrast with those of Sheehan-Dare *et al.* This conflicting evidence is difficult to explain. Possibly, acne protects against haematological malignancies but not against solid tumours. Another reason may be the different age distribution of the patients (15–39 years in the study of Sheehan-Dare *et al.* vs. 25–50 years in our study). Long time lapses since puberty or adolescence may obscure reliable acne histories. Thirdly, the findings of Sheehan-Dare *et al.* may merely represent a chance observation without clinical relevance. Further studies are indicated to settle the controversy.

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Reticulate hyperpigmentation distributed in a zosteriform fashion

SIR, We were interested in the recent report by Iijima *et al.* entitled 'Reticulate hyperpigmentation distributed in a zosteriform fashion: a new clinical type of hyperpigmentation'.[1] We agree that this is basically not different from 'Progressive cribriform and zosteriform hyperpigmentation' (PCZH) described by Rower *et al.*,[2] although the latter is characterized by a later onset and by a distribution limited to one dermatome.

We recently observed a case of acquired hyperpigmentation which showed features common to both disorders. A male aged 7 first developed a cribriform light brown pigmented linear lesion on the right upper chest at the age of 3. A second arcuate hyperpigmented lesion, formed by two parallel lines, occurred on the right middle abdomen at the age of 6. The lesions enlarged progressively in a zosteriform distribution, following the Blaschko's lines. There was no history of injury and no symptoms. The hyperpigmented areas showed no atrophy or hyperkeratosis of the skin surface and no hypertrichosis was noted. The nails, the hair and the oral mucosa were normal. Physical examination revealed that the right side of the body (ipsilateral to the pigmentation) was slightly larger than the left. Neurologic and ophthalmologic examinations did not show any abnormalities.

As in Iijima's case of reticulate hyperpigmentation the hyperpigmented areas in our patient appeared during the first decade of life. However, the distribution of the lesions spread to two ipsilateral dermatomes, unlike both PCZH which is limited to one dermatome and reticulate hyperpigmentation which has a generalized distribution. We believe this case is a variant of both disorders, supporting the conclusion that reticulate hyperpigmentation can be regarded as multiple lesions of PCZH.[1] We also suggest that PCZH may be considered as a particular type of Becker's naevus. This is classically described as an acquired unilateral hyperpigmented macule with hypertrichosis. However Becker's naevus may be bilateral and multiple and may have a zosteriform pattern,[3] and hypertrichosis may not always be present.[4] The histological findings in Becker's naevus and PCZH are similar, although Becker's naevus can also be associated with underlying hypoplastic or hyperplastic abnormalities [3,5,6] as was observed in our patient.

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British Journal of Dermatology (1989) **121**, 281–284.

Book Reviews

Advances in Dermatology. Vol. 4. Edited by J.P.Callen, M.V.Dahl, L.E.Golitz, L.A.Schachner and S.J.Stegman (1989). Chicago, London, Boca Raton: Yearbook Medical Publishers Inc. Pp. 374. ISBN 0-8151-1417-6. Price £37.50.

The stated goal, for this whole series, is 'to publish in a timely manner state-of-the-art information relating to our ever changing speciality'. This has been achieved admirably.

The layout is clear, the 15 articles being grouped under five parts. Each contribution is well referenced and has an editorial comment. (Some might consider such comment, though germane, more suited to the standard Year Books.) The list of contents, the illustrations and tables, and the index are beyond reproach.

Part 1 consists of a thorough and practical review of cyclosporin A, which is excellent; a rather lightweight but topical survey of sports-related skin injuries; and a concise and most helpful discussion on pyoderma gangrenosum and related disorders.

The second part provides a thought provoking review of endemic pemphigus foliaceus, an up-to-date discussion on the role of calcium as a second messenger and, the only British contribution, an elegant review of dermatitis in food handlers.

In part 3 are found a paper on the Miami experience of the changing pattern of pyoderma in childhood, a dissertation on the psychological aspects of skin disease in the several stages of childhood, and a detailed, instructive review of the mast cell and mastocytosis in childhood.

The dermatopathology part contains the largest and most comprehensive review in the book—on vascular skin neoplasms: this surely will become a much used reference. The article on the widening spectrum of human Lyme disease is fascinating, but the challenging overview of keratinocyte differentiation reveals a complex subject. The final review of part 4 deals with the histological changes in alopecia, of which there are notable illustrations.

The two articles in the final part, 5, concern dermatological surgery, dealing with progress in the quest for synthetic skin, and with the inter-disciplinary difficulties of analysing the meaning and significance of tissue margins.

There is much in this elegant book to interest all the readership, and every dermatological library should possess a copy.

J.N.S.Mitchell

Advanced Geriatric Medicine 6. Edited by F.I.Caird and J.Grimley-Evans (1987). Bristol: Wright, Pp. 188. ISBN 0-7236-0651-X. Price £25.00.

I enjoyed reading this slim indexed hard-back volume which is one of a series reviewing topics of relevance to the care of the elderly (i.e. over 65 age group).

It has 19 contributors, most of whom work in Scotland. It is divided into four parts, namely, demography, dermatology, therapeutics, and future developments. Maps, charts, graphs and statistics are plentiful in the non-dermatological sections and updated chapters on adverse drug reactions, hypothermia and choice of hypoglycaemic drug are always important.

The dermatology section occupies one-third of the book and contains the 17 monochrome illustrations. It is divided into five well-chosen chapters, namely, biology of ageing skin (Finlay), cutaneous infections (Cox and Roberts), blistering diseases (Harrison and Simpson), cutaneous malignancy (MacKie), and modern dressings for leg ulcers (Varghese and Carter). All are interesting and useful but a practical problem with modern dressings is that many are unavailable on NHS prescriptions. Both pemphigus foliaceus (p. 101) and Dubreuilh (p. 124) were wrongly spelt and the concentration of gamma benzene hexachloride (p. 98) should surely be 1%.

The intended readership includes junior hospital doctors and recently appointed geriatricians. With this readership in mind, the text suits admirably. It is a good book for the non-dermatologist, and the price is just about right.

Dermatological Signs of Internal Disease. Edited by J.P.CALLEN, J.JORIZZO, K.E.GREER, N.PENNEYS, W.PIETTE AND J.Z.ZONE. (1988) Philadelphia: W.B.Saunders and Co. Pp. 383. ISBN 0-7216-1860-X. Price £34.50.

General physicians and general practitioners are aware that subtle dermatological signs may herald significant changes within the patient and it is to these non-dermatologists that this book is aimed. Written by six dermatologists, practicing academic dermatology in the southern United States, they state in their preface that the book is intended for primary care physicians and only in a small way for the trainee dermatologist.

In the tradition of medical textbooks and medical teaching, the book has been arranged according to systems. The broad section headings include rheumatology, general dermatological reactions to systemic disease, haematology and oncology, endocrine and metabolic, gastroenterology, infections and finally a pot-pourri of other systemic diseases with cutaneous manifestations. There is a very concise appendix on drug reactions and the skin.

There is considerable variability in the quality of the text between the six authors. In a book aimed at general physicians some of the authors have put in too much detail about the general medical condition at the expense of a better description of the dermatological manifestations. The general physician looking for an indication as to whether a cutaneous sign could be part of his patient's condition, does not require a long description of the medical condition.

The chapters on cutaneous gastroenterology and cutaneous infection are particularly well written, they adhere to the aim of the book giving good descriptions of the dermatological manifestations of a particular systemic disease. Naturally the emphasis is on diseases seen in the geographical area of the authors so some of the cutaneous infections will have little or no relevance to practitioners in the U.K. and the rest of Europe.

The book has many black and white photographs, which are unfortunately quite small, and consequently much detail is lost. Eight pages of colour plates are all repeats of black and white pictures already in the text. The printing method used has rendered the pictures a most vivid vermilion.

The general physician faced with a patient with a dermatological sign has few texts to refer to. This book goes some way to filling that need but with an understandable eye to the expense of the book good colour illustrations have been neglected. Nevertheless this would be a useful book for a general physician or general practitioner who wants help in identifying the manifold cutaneous changes associated with systemic illness.

S.I.WHITE

Pseudoxanthoma Elasticum. Clinics in Dermatology, 6, 1. Edited by L.C.PARISH AND J.T.CRISSEY (1988) Philadelphia; J.B.Lippincott. Pp. 159. Price £83.00.

This single author volume summarizes the 10-year experience of 100 dermatologically referred patients with classical biopsy proven pseudoxanthoma elasticum (PXE) examined and studied in detail at the Technical University Health Sciences Centre in Lubbock, Texas. All patients were examined by Dr Neldner and followed up by him with the exception of detailed ophthalmological examination by various ophthalmologists who assessed retinal changes using retinal photography and angiography. Other investigations included various parameters of peripheral vascular function, neuropsychological assessment, pulmonary function studies, dietary calcium levels, serum lipid and triglyceride levels and various light and electron microscopical examinations and attempts to identify prognostic criteria with the extent of skin, eye and vascular changes and ends with chapters on aetiology and treatment.

As a guide to the general clinical features, diagnosis, complications and history of the disease this is an excellent well illustrated account and summarizes succinctly every conceivable feature that a working dermatologist could desire. The accounts of the clinical genetic aspects and possible dietary effect of calcium are more controversial. Quite clearly Dr Neldner has found a very different distribution of autosomal dominance compared with recessive inheritance than we did with only a 3% incidence of dominant transmission in his series compared with our 10% for typical cutaneous PXE and approximately 40% for the loose jointed atypical variant. Random genetic markers (restriction fragment length polymorphisms) for the suspected candidate genes will of course settle the dispute in suitable families. Thus, in classical recessive families with multiple affected siblings, homozygous or double heterozygous assortments of the parental markers will occur, whereas in the putative dominant families assortment of the candidate gene markers should both confirm and identify affected heterozygotes and sometimes indicate affected but doubtful patients.

Dr Nelders's chapter on ultrastructural electron microscopical changes in PXE contains some intriguing clues which suggest that collagen fibril abnormalities have a fundamental role in some forms of PXE rather than the secondary role that he suggests. Chapter 18 covers simple genetic counselling, a brief account of plastic surgical results, sensible precautions for eye care and a simple guide to the management of gastro intestinal bleeding (here Dr Neldner's experience differs from ours as we observed specific gastric lesions such as haemangiomas and microaneurysms as well as diffuse haemorrhage. Treatments such as vitamin E, corticosteroids, diphosphonate and various chelating agents such as EDTA and zinc are mentioned but have no place in the management of PXE.

This book is a useful addition to the accounts in major textbooks of dermatology and is essential reading for dermatologists, ophthalmologists, general physicians, surgeons, gastroenterologists and connective tissue researchers. Whilst reluctant to recommend its purchase to all individual members of these groups, I suggest it a worthwhile buy for their relevant departmental and main hospital libraries, despite its high recommended price of £83.00.

F.M.Pope

Textbook of Dental Pharmacology and Therapeutics. J.G.Walton, J.W.Thompson and R.A.Seymour. (1989) New York: Oxford University Press. Pp. 486. ISBN 0-19-261235-2. Price £22.00.

This first edition is basically an expanded version of a previous smaller textbook written by two of the authors entitled *Pharmacology for the Dental Practitioner*. The book is divided into 25 chapters beginning with 'General principles of drug action' and finishing with 'Therapeutic measures for common oral conditions'. There is a great deal of fairly detailed pharmacokinetics in the initial chapters and at this point I was happy to have an honours science degree in pharmacology. The authors have provided much practical information essential for modern dental practice, and the sections on pain appreciation are particularly clear and up-to-date. Trade names are given as well as generic names when discussing drugs and the cardiovascular system, and this is of practical significance as often practitioners (and dental students) find it difficult to take the step from appreciating drug mechanisms to knowing which drugs the patients are taking.

It is inevitable, since at present there is no national concensus on how to treat a variety of oro-facial diseases, that the therapeutic aspects of the book could be faulted. However, the authors have attempted to play safe and although the treatments suggested are sketchy, they are certainly not too contentious.

P.J.Lamey

Transdermal Drug Delivery: Developmental Issues and Research Initiatives. Edited by J.Hadgraft and R.H.Guy (1989) New York: Marcel Dekker, Inc. Pp. 324. ISBN 0-8247-7991-6. £119.50.

This book is volume 35 in the series Drugs and the Pharmaceutical Sciences and the subsidiary title gives a good indication of the approach adopted to the subject of transdermal drug delivery, which is almost to provide a *modus operandi* for the development of transdermal formulations. The price perhaps reflects the interest in this book which will come from the pharmaceutical industry and their ability to pay for it. Of the 18 contributing authors, six come from the pharmaceutical industry and eight from university departments of pharmacy, so that overall there is a definite pharmaceutical emphasis. There are 12 chapters and chapter three, by the editors, deals with the selection of candidate drugs for transdermal delivery and is essentially the core of the subject matter, the other chapters covering particular aspects in much greater detail. The transdermal route offers an attractive means of drug administration, avoiding as it does, the vagaries of the gastro-intestinal tract and the inconvenience of the parenteral route. However, the success of this approach implies subversion of what is now recognized to be a rather sophisticated barrier resident in the stratum corneum and in the underlying viable epidermis. The first two chapters provide a good review of the biology and biochemistry of the stratum corneum, which is the acknowledged rate limiting barrier to the diffusion of drugs, and the nature of the barrier function of this tissue against the ingress of topically applied agents. Several chapters describe the various strategies which may be used to enhance and control penetration of drugs through the skin; by means of delivery systems such as dermal patches, chapter seven, penetration enhancers, chapter ten and the undervalued technique of iontophoresis, chapter eleven. There is a good review of *in vitro* methodology for evaluating transdermal delivery, chapter nine and in chapter eight an interesting treatment of transdermal drug delivery in neonates, who by virtue of body size and skin permeability are good subjects for this therapeutic approach. Although there is a pervasive enthusiasm for the transdermal route, which one might expect, this book is also realistic and I was particularly pleased to see the problems of drug metabolism in the skin, chapter five and on the skin by micro-organisms, chapter six, addressed and in particular the problems of allergic and irritant responses to topical agents described in chapter four. The immune response of the skin may be the most difficult of its barrier functions to overcome. The last chapter provides a rare review of the materials used to construct transdermal delivery devices.

This is an expensive but none the less excellent book which provides a timely 'pulling together' of the several diverse aspects of this subject and although aimed primarily at the pharmaceutical scientist contains much to interest those with a more clinical orientation to dermatology. The application of drugs to the skin in any type of formulation for a local or systemic effect is within the ambit of this book. There are some minor typographical errors which tend to annoy and an abysmal level of geographical knowledge which places Loughborough in Scotland!

A.J.BAILLIE

Books Received

Benchmarks: Alternative Methods in Toxicology. Edited by M.A.MEHLMAN (1989) Princeton: Princeton Scientific Publications.

Martindale: The Extra Pharmacopoeia. (29th Edition) (1989) The Pharmaceutical Press. Pp. 1930. Price £95.00.

Contact Dermatitis in India. J.S.PASRICHA. 2nd edn (1988) New Delhi: The Offsetters. Pp. 155.

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News and Notices

Aspen Summer Skin Seminar, 11–14 July 1989, Aspen Colorado

A seminar will be held to update the practising dermatologist in a wide variety if topics of practical interest. In addition to lectures concerning pathogenesis and treatment of cutaneous infections, inflammation and cancers, three sessions will be devoted to reviewing interesting and challenging cases.

For further information contact: Office of Continuing Medical Education, 4200 East 9th Ave., Box C295, Denver, CO 80262, U.S.A.

An International Update in Dermatology and Dermatopathology: 2–5 September 1989

This international meeting, jointly organized by the Pathological Society of Great Britain and Ireland and the British Society for Dermatopathology will he held at The Dome, Brighton. It is designed to provide pathologists and dermatologists with a state of the art review of dermatopathology. Over 3 days lectures and seminars will be given by a panel of invited experts. Slide seminars will be presented by Dr R.Reed, New Orleans and Professor A.B.Ackerman, New York. Abstracts are invited for poster and oral presentations. A full social programme is included. Further details from: Mrs L.Barducci, British Association of Dermatologists, 6 St. Andrew's Place, Regent's Park, London NW1 4LB (Tel. 01 935 8576).

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Rapid diagnosis in varicella and herpes zoster: re-evaluation of direct smear (Tzanck test) and electron microscopy including colloidal gold immuno-electron microscopy in comparison with virus isolation

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SUMMARY

The Tzanck test and electron microscopy with the technique of colloidal gold labelling in varicella-zoster virus (VZV) infections were compared with virus isolation in 54 patients with clinically suspected varicella or herpes zoster infection. The Tzanck test and direct electron microscopy can determine whether or not an eruption is herpetic but cannot distinguish between herpes simplex virus (HSV) and VZV infection. However, colloidal gold immuno-electron microscopy, using monoclonal antibodies against HSV and anti-VZV IgG, can distinguish between these two herpes viruses. This achieves the same specificity as virus isolation followed by virus neutralization or virus typing using immunofluorescence techniques. The Tzanck test was positive in 91%, virus isolation, under optimal conditions of sampling and transportation, in 80%, direct electron microscopy (negative staining) in 80%, and colloidal gold immuno-electron microscopy after a virus concentration procedure in 95% of the cases. The colloidal gold technique offers a rapid diagnosis in patients with suspected VZV infection.

In most instances, varicella and herpes zoster can be readily diagnosed. In difficult cases the confirmation of a VZV infection by laboratory tests may be necessary; the commonly applied technique of virus isolation of VZV is specific, but not so good when compared with the isolation

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Correspondence: Dr E.Folkers, Department of Dermatology, Hospital 'de Heel', Postbox 210, NL-1500 EE Zaandam, The Netherlands of HSV virus. The isolation of VZV is only reliable under optimal conditions of sampling, transportation and storage but needs days and sometimes weeks to confirm.[1] Counter immunoelectrophoresis, immunofluorescence, and ELISA techniques can also differentiate between HSV and VZV infection.[2,3,4] Monoclonal antibodies to VZV antigens are becoming more available, but as yet are not widely used.[5]

A rapid and easy additional procedure is the cytopathological diagnosis, that can support clinical diagnosis of herpetic infection and is based on the Tzanck test. This test is diagnostic for HSV and VZV, but cannot differentiate between the two infections.[6,7] Solomon *et al.*,[1] in their study of the applicability of the Tzanck test in comparison with virus isolation, used the clinical diagnosis of VZV infection as a standard reference, but the vesicular appearance and dermatomal distribution of the herpetic eruption cannot provide a sufficient basis for making a clear differentiation between HSV and VZV infection.[8] Even virus isolation of HSV does not reach 100% sensitivity in typical cases.[9] Therefore, virus isolation cannot be accepted as the only verification standard in detecting or excluding VZV infection. Clearly, there is a need for a rapid supplementary tool for revaluating the Tzanck test in clinical diagnosis of VZV infection. This can be obtained by colloidal gold immuno-electron microscopy, which differentiates between HSV and VZV.[10]

We present the results of a study that illustrates the usefulness and reliability of the Tzanck test with direct electron microscopy (negative staining) and colloidal gold immuno-electron microscopy as compared with virus isolation.

METHODS

Patients

Samples were collected from skin lesions of 54 patients, during the period from December, 1984 to March, 1986. Eleven of the patients, suspected of having a VZV infection, were attending the out-patient department of Pediatric Dermatology or were hospitalized in the Sophia Children's Hospital Rotterdam (SCH), and 43 were attending the out-patient department of Dermatovenereology or were hospitalized in Hospital 'de Heel' in Zaandam (HdH). The lesions were classified as follows: maculopapular (MP), vesicular (V), pustular (P) and/or crusted (C). The SCH-group and HdH-group together had 11 patients (aged 0–10 years) clinically suspected of having varicella and 43 patients (aged 2–86 years) with symptoms of herpes zoster.

Virus isolation

The vesicular or pustular lesion was opened with a scalpel blade and the fluid collected on a swab which was then transferred immediately to a viral transport medium. In the case of crusted lesions, the crusts were removed and a swab taken from the erosion. With maculopapular lesions, the affected skin was scraped intensively with the edge of the scalpel blade to obtain a superficial erosion and from this a swab was taken.

Swabs obtained from patients of the SCH-group were inoculated in 3 ml transport medium (Dulbecco's modification of Eagle's medium (DMEM) with 10% fetal bovine serum and antibiotics). Within 0·5 h after collection the samples were inoculated into tube cultures of HEL (Human Embryonic Lung) fibroblasts (0·2 ml/tube, 2 tubes/specimen). Virus isolation was attempted at 37°C for a maximum of 2 weeks and cells were scored daily for a cytopathic effect (CPE). Identification of isolated viruses was performed in an immunofluorescence test using monoclonal antibodies against herpes simplex viruses and a polyclonal antiserum against VZV

(J. Choufoer-Habova, Department of Virology, SCH). In case of negative results, a second passage was performed.

Swabs obtained from patients of the HdH-group were inoculated in 3 ml transport medium (Hank's balanced salt solution (HBSS) with 0·5% gelatin, sodium bicarbonate to adjust pH to 7·2, and antibiotics) and transported at room temperature within 24 h to the Department of Virology, GG&GD Amsterdam (Head: Dr R.A. Coutinho). If transportation of the specimen could not be done within 24 h, the sample was inoculated into the transport medium, stored at −80°C within 0·5 h after collection, and then transported afterwards in this frozen condition to the laboratory. The samples were then immediately inoculated into cultures of HEL fibroblasts. The cells were examined for the presence of VZV specific CPE. If no CPE was obtained after 3 weeks of culturing, a second passage was done for another 3 weeks.

Tzanck smear test

Scrapings from the base of the vesicles, pustules, or erosions, were smeared on to a glass slide and air-dried. The material was fixed in methanol and stained within 0·5 min with Hemacolor® (Merck). The slide was dipped five times in methanol, three times in eosin, and three times in thiazine. The slide was then rinsed with water and air-dried and a permanent mounting medium and coverglass applied. The smear was then examined under light microscopy.[11]

Epidermal cells were scored for characteristic nuclear changes as seen in herpetic infections. These nuclear changes included enlargement, multi-nucleation and crowding of the nuclei with nuclear 'moulding'. Different stages of peripheral margination of the nuclear chromatin were seen, and the ground substance of the nucleus was more coarse or showed an opaque appearance (ground glass aspect). In addition, mononuclear, non-giant cell virocytes were recognized on the basis of the nuclear changes described above. Sometimes intranuclear inclusions, surrounded by a prominent halo, were observed.[7,12] All preparations were examined by one of the authors and were then examined 'double-blind' by two others.

Electron and immuno-electron microscopy

After obtaining samples for virus isolation and for the Tzanck test, the lesion, or an adjacent one was scraped intensively with the curved side of a vaccinostyle, fixed with the scarification side in the hollow of the cap of a plastic sampling tube. The cap was replaced on the tube which contained a small moistened gauze. Within 0·5 h after sampling, the material was frozen to −25°C in a normal freezer and transported in frozen condition immediately or within 2 weeks to the laboratory for electron microscopy. At the laboratory, the sample was frozen to −80°C, if storage for a longer period was necessary. Without prior knowledge of the clinical appearance, the results of the Tzanck test or virus isolation, identification of the virus by electron microscopy (EM) and virus typing by colloidal gold immuno-electron microscopy (CG-IEM) was carried out.[10]

The CG-IEM technique can be described briefly as follows. The sample was homogenized in a mortar with sterile sand and diluted with 10 mM Tris-HCl (pH 7·3) to about 500 μl. The sand was pelleted by low-speed centrifugation in a Beckman desk microfuge (maximum speed, 3 s). The virus particles present in the supernatant were adsorbed to the carbon side of collodion-carbon-coated nickel grids (10 min) and stained with 2% phosphotungstic acid (pH 6·8). Virus identification was performed in a Philips EM 300 electron microscope at a voltage of 80 kV. If no virus particles were detected, virus concentration was carried out by centrifugation of the remaining supernatant through a 70 μl 30% potassium tartrate cushion in a Beckman SW 50 rotor (32 000 *g*, 30 min.) The pellet was suspended in 20 μl 10 mM Tris-HCl and sonicated in

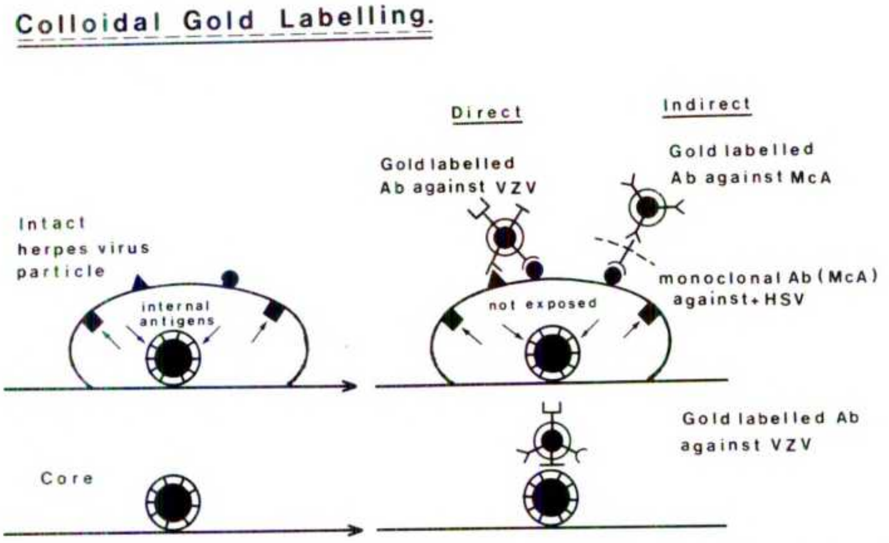
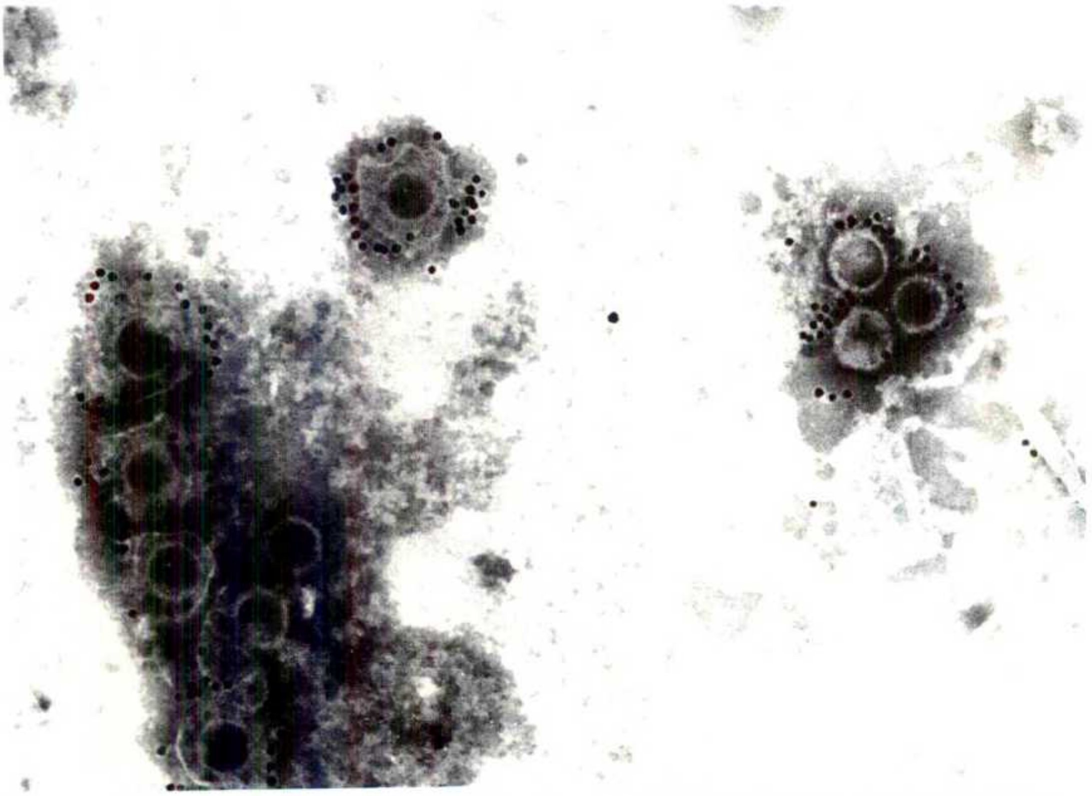

Colloidal Gold Labelling.
Direct
Indirect
Gold labelled Ab against VZV
Gold labelled Ab against McA
Intact herpes virus particle
internal antigens
not exposed
monoclonal Ab (McA) against+HSV
Gold labelled Ab against VZV
Core

FIGURE 1. Schematic representation of colloidal gold labelling steps used in this study. A direct labelling procedure was used for identification of VZV envelopes and cores. An indirect labelling technique was applied to differentiate VZV from HSV.



FIGURES 2 and 3. Gold-tagged polyclonal antibodies against VZV labelled both viral envelopes (Fig. 2) and viral cores (Fig. 3) (× 60 000).

closed tubes in a cuphorn attachment of a Branson sonifier.[10] Virus adsorption, staining, and virus identification were done as described above.

After virus adsorption, the grids were incubated with highly specific gold-tagged, polyclonal antibodies against VZV (showing no cross-reactions with envelopes and cores of HSV, cytomegalovirus, and Epstein–Barr virus) for 1 h at room temperature (Fig. 1). The preparation and the specificity of the gold-tagged antibodies have been previously discussed.[10] After incubation, the grids were thoroughly washed with PBS and stained with phosphotungstic acid. The gold particles of the anti-VZV gold conjugate can be seen as electron-dense granules around cores and enveloped particles (Figs 2 and 3). If no reaction with the anti-VZV gold conjugate was seen, HSV typing was carried out using monoclonal antibodies in the first step and gold-tagged, rabbit-anti-mouse antibodies in the second step (Fig. 1).[10]

Statistical analysis

McNemar's test[13] was used to compare positive and negative scores obtained from the Tzanck tests as determined by different investigators (EF, APO and JND).

RESULTS

Fifty-four patients were selected on the basis of clinical symptoms of VZV infection. Laboratory tests, however, demonstrated herpes virus infection in 51 patients. The clinical diagnosis 'VZV infection' could not be maintained in three cases. One child with a late stage of varicella had pustules and crusted lesions and the diagnosis has been changed to impetigo. One child had an impetigo with pustules and a localized form of staphylococcal scalded skin syndrome (SSSS). The other patient was suspected as having an early maculopapular stage of VZV infection and on skin biopsy a vasculitis of unknown origin was demonstrated. Here, serological examination did not demonstrate anti-VZV IgM. Forty-six patients showed VZV infection confirmed by virus isolation and/or CG-IEM and in five patients HSV infection was demonstrated.

TABLE 1. Patients with proven VZV infection by virus isolation and/or CG-IEM, in two separated populations ($n=46$)

	Lesion type	Number of patients	Virus isolation		CG-IEM	
			+VZV	−	+VZV	−
Sophia Children's Hospital	MP	3	2	1	3	0
	V	6	5	1*	5	1*
	P	1	1	0	1	0
Total		10	8	2	9	1
Sensitivity (%) of virus isolation under optimal conditions, 80%						
Hospital 'de Heel'	MP	3	0	3	2	1†
	V	21	4	17	21	0
	P	12	1	11	12	0
Total		36	5	31	35	1
Sensitivity (%) of virus isolation under suboptimal conditions, 14%						

MP = maculopapular.
V = vesicular.
P = pustular.
* = not the same patient.
† = EM positive afterwards in vesicular stage.

TABLE 2. Patients, clinically suspected of having VZV infection, with proven HSV infection by virus isolation and/or CG-IEM ($n = 5$)

Hospital 'de Heel' ($n = 5$)	Lesion type	Number of patients	Virus isolation +	HSV −	CG-IEM VZV	HSV
	P	4	2	2	—	4
	C	1	0	1	—	1

P = pustular.
C = crusted.

Virus isolation

The results of virus isolation obtained in the two investigated populations (SCH and HdH-group) are shown in Tables 1 and 2. In the SCH-group ($n = 11$), virus isolation was done under optimal circumstances. VZV infection was confirmed in 10 patients by virus isolation and/or CG-IEM. Virus isolation was positive in eight patients (80%, Table 1). In the HdH-group ($n = 43$), virus isolation was carried out under suboptimal circumstances (see Methods). Although VZV infection was proven by CG-IEM in 36 patients (including one patient with a positive CG-IEM score afterwards in the vesicular stage of infection), virus could be isolated from no more than five patients (14%, Table 1). In five out of 54 patients with zosteriform herpetic eruptions, HSV was demonstrated in two patients by virus isolation and by CG-IEM; from the other three patients no HSV positive virus isolation could be obtained, but CG-IEM confirmed the presence of HSV particles (Table 2).

Tzanck smear tests

The results of Tzanck smears are listed in Tables 3–5. Agreement between the observers was

TABLE 3. Comparison of the Tzanck test scores, obtained by different investigators, in proven VZV infection by virus isolation and/or CG-IEM ($n = 46$)

		Number of positive results Tzanck smear			EM	
		Investigators				
Lesion type	Number of patients	EF	APO	JND	Direct	CG-IEM
Maculopapular	6	5	5	6	2	5
Vesicular	27	26	26	26	23	26
Pustular	13	11	12	12	12	13
Total number:	46	42	43	44	37	44
Sensitivity (%)		91*	93*	95*	80	95

* Using McNemar's test no significant differences were obtained between the Tzanck scores of the investigators ($P > 0.1$).

TABLE 4. Comparison of the Tzanck test scores, obtained by different investigators, in patients with a proven HSV infection by virus isolation and/or CG-IEM, clinically suspected of having VZV infection ($n = 5$)

			Tzanck test					
		Investigator:	EF		APO		JND	
Lesion type	Number of patients	Score:	+	−	+	−	+	−
Pustular	4		3	1	3	1	3	1
Crusted	1			1		1		1

TABLE 5. Results of Tzanck tests in patients with clinically VZV infection, without proven herpetic infection ($n = 3$)

		Results of Tzanck test obtained by investigator		
Diagnosis	Lesion type	EF	APO	JND
Impetigo	pustular	−	−	+
Impetigo with local SSSS	pustular	−	−	−
Vasculitis	maculopapular	−	−	−

SSSS = staphylococcal scalded skin syndrome.

obtained in 44 Tzanck preparations out of 46 proven VZV infections (42 were considered positive and two negative by all three investigators). There was disagreement between the observers in two cases, involving an early (maculopapular) and a late (pustular) stage of VZV infection. Investigators EF, APO and JND achieved a sensitivity of 91, 93 and 95%, respectively. Using McNemar's test, statistical analysis of the Tzanck test sensitivity, obtained by these investigators, showed no significant differences (Table 3). Agreement was obtained in five Tzanck preparations of proven HSV infections (Table 4): three were scored positive and two negative by all three investigators.

Table 5 summarizes the Tzanck readings of three patients without proven herpetic infection. The observers disagreed in a Tzanck test reading of one child, suspected of having a late stage of varicella with pustules and crusted lesions. Virus isolation and CG-IEM were unable to prove herpetic infection in this case and it was finally diagnosed as impetigo.

EM and immuno-electron microscopy

In the investigated population ($n = 54$), herpes virus was detected in 40 patients by EM, carried out directly on homogenates of the material obtained from the skin lesions. After a virus concentration step, herpes virus was demonstrated in 49 patients.

The results of EM and CG-IEM in comparison with the Tzanck test are listed in Table 3 and 4. With CG-IEM, varicella-zoster virus was found in 44 cases, and HSV type 1 could be identified in five cases. In one patient with herpes zoster, Tzanck test, virus isolation, and EM examination were negative in the early maculopapular stage; a positive result with CG-IEM was obtained afterwards in the vesicular stage of the infection.

DISCUSSION

Vesiculobullous eruptions are known to occur during the course of several viral infections, including herpes simplex, herpes zoster, vaccinia (and variola), hand-foot-and-mouth disease and other enteroviral infections. The occurrence of vesicles and vesiculobullous lesions in cytomegalovirus infection has been described,[14,15] and a congenital vesicular eruption due to *Haemophilus influenzae* type B has been observed.[16] Other diseases may also be confused with varicella and herpes zoster.[17,18] However, in most patients a VZV infection can be diagnosed from the typical clinical features.

Disseminated herpes simplex may occasionally resemble varicella. Zoster-like cutaneous manifestations can develop due to HSV infection. Kalman and Laskin[8] reported that 13% of their patients with the clinical diagnosis herpes zoster actually had herpes simplex infections with a facial distribution in four of these six patients. In another report 10% of patients with clinical impression of herpes zoster, actually suffered from HSV infection as demonstrated by virus isolation.[1] In our study we found that 9% of the patients, clinically suspected of VZV infection, actually had HSV infection. This was demonstrated by virus isolation and CG-IEM. Three of these patients had a facial distribution of their rash and one had involvement of dermatome T12. One child had a generalized varicelliform eruption of HSV infection.

VZV is less tolerant than HSV to transport and storage conditions and the sensitivity of virus isolation in varicella infections at mainly the vesicular stage was 63%. For herpes zoster a sensitivity of 70% in the vesicular stage was obtained and a 40% sensitivity in the pustular stage. In the study of Kalman and Laskin[8] the sensitivity of VZV isolation was 43%. In our previous study,[19] we obtained a sensitivity of virus isolation in varicella of 60% and a sensitivity of virus isolation in five patients with herpes zoster of 100%. In the present study we focused attention on two groups of patients. The first group showed that virus isolation of VZV under optimal conditions could reach a sensitivity as high as 80%. In the second group, representative for the general practitioner, the sensitivity of virus isolation dropped to 14%. Diagnosis on the basis of virus isolation takes several days because the VZV proliferates at a slower rate than HSV. Virus isolation is less suitable for rapid diagnosis.

The Tzanck test has been used for a long time as an aid to confirm the diagnosis of cutaneous and mucosal herpesvirus infections. The typical cytopathological changes in herpesvirus-infected keratinocytes can easily be recognized in early, intact vesicles and pustules. In the very early stage of a herpetic infection the cytological changes can be subtle or equivocal and in the older pustular lesions the necrosis of the infected keratinocytes is obvious and the nuclear details may be unclear. It should be noted that the Tzanck test is not specific for VZV infection but the test can reveal whether or not an eruption is herpetic. Although lesions of VZV show less cell destruction and inflammation in the early stages of infection than those of HSV, these features cannot be used in differential diagnosis.[7,12] In the present study the sensitivity of the Tzanck test was as high as 90%. Statistical analysis showed no significant differences of Tzanck test sensitivity, obtained by different investigators. The results were largely based on verification with the new immunogold EM technique. A diagnosis of VZV infection can be obtained within 3 h and of HSV infection in 4 h.[10] This technique scored higher than did virus isolation of VZV under optimal conditions. Another advantage of EM is its reduced sensitivity to transport and storage conditions and for this reason, colloidal gold virus typing was used in this study as the standard verification method for the Tzanck test.[10]

Our current results confirm previous conclusions[1] that the Tzanck test is valuable in supporting the clinical diagnosis of VZV infection. It is inexpensive, easy to perform and rapid, but experience and training are required. Proper interpretation of Tzanck tests should not lead to misdiagnoses. Cytopathological re-screening may be an important supplementary diagnostic procedure.[1,19]

Rapid diagnostic techniques in herpetic infections may be necessary for the decision to start antiviral therapy, particularly in cases involving immuno-suppressed or immuno-compromised patients, or in neonatal herpes simplex and neonatal varicella. Since VZV and HSV have varying sensitivity to different antiviral agents, such as acyclovir, early differentiation between these herpes viruses is desirable.[8] In conclusion, this study confirms that the Tzanck test is rapid and reliable in VZV infection, but cannot differentiate between VZV and HSV. Immunofluorescence cytology using monoclonal antibodies, which are now becoming more available, should eliminate this problem in future.[5,20] None the less, the Tzanck test will continue to be important in rapid diagnosis of herpetic skin infections. EM, after a virus concentration step, is another rapid and reliable test in VZV infection. However, the classical EM technique also cannot differentiate between VZV and HSV. CG-IEM provides the possibility for rapid and specific diagnosis in herpetic infections.[10]

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The use of A-scan ultrasound in the assessment of small skin tumours

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SUMMARY

This study describes the use of A-scan ultrasound for the measurement and characterization of tumour tissue in small skin tumours—basal cell carcinoma, melanocytic naevus, hypertrophic scars and intraepidermal epithelioma. Assessment of the A-scan traces by measurement of: (i) the amplitude of echoes within the area of interest; (ii) the density (number per unit depth) of these echoes; (iii) the regularity of spacing and amplitude of the echoes, and (iv) the amplitude of echoes beneath the area of interest, was used to assist in the differential diagnosis. The results show significant differences in echo amplitude between all the tumours and normal skin. When the tumour A-scan traces were analysed the results indicated that the best discriminating feature between tumours was that of amplitude, followed by regularity, density, non-normalized thickness and finally amplitude of echoes from beneath the tumour.

Ultrasound-derived skin thickness measurements were compared to histological measurements for the lesions, and an excellent correlation was found ($r = 0{\cdot}96$). It was considered that the A-scan ultrasound investigation of small tumours of the skin was able to provide information on the nature of the tissues contained and may assist in their differential diagnosis.

In its application to the investigation of skin, ultrasound has been used almost exclusively for the measurement of skin thickness[1–3] and characterization of the depth of tumour invasion.[4,5] However, if lesions or abnormal skin conditions are classified by changes in the composition and structure of their tissues, then A-scan examination should reflect these changes, since echo amplitudes and spacings are determined solely by these properties. We believe that this information contained in the A-scan trace is of diagnostic significance, and we have undertaken the present study of normal and abnormal skin to try to characterize the echographic features of the resulting traces and develop from them appropriate parameters.

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METHODS

Subjects

Patients with basal cell carcinomas (BCC), dermatofibromas, hypertrophic scars, moles and intraepidermal epitheliomas (IEE) were studied. The A-scan traces from dermatofibromas and hypertrophic scars are similar and are included under a single heading of 'fibrosis'. Each lesion was diagnosed clinically and the diagnosis confirmed histologically by biopsy and/or subsequent removal.

Ultrasound measurements were made from the tumour and from adjacent normal skin. Patient details were as follows: the BCC group consisted of 20 patients with a mean age of 64·5 years (age range 50–80); the 'fibrosis' group had 11 patients with a mean age of 45·2 years (age range 21–62); the mole group had 10 patients with a mean age of 37·4 years (age range 23–53); and the intraepidermal epithelioma group had five patients, whose mean age was 65·8 years (age range 60–71). Patients gave their informed consent and the study received approval from the Joint Ethics Committee of the South Glamorgan Area Health Authority/University of Wales College of Medicine.

Ultrasound equipment

A pulsed A-scan generator with wide bandwidth and low noise receiver (the Dermal Monitor 70, Cutech Ltd., High Wycombe, U.K.) was used in conjunction with a plastic film (polyvinylidene di-fluoride (PVDF)) transducer. This system generates acoustic pulses of 0·1 μs duration, 18 MHz centre frequency and 16 MHz bandwidth, which allows a perceived resolution on the A-scan trace of approximately 50 μm. The focused transducer is coupled to the skin via a water column in a perspex cone which ensures that the skin is at a reproducible axial position and enables the operator to maintain the beam incident angle at 90 degrees. Each trace is displayed on a calibrated oscilloscope screen which is photographed with a Polaroid camera to allow rapid examination of the captured trace and a permanent record for subsequent analysis. All data presented below were obtained from such Polaroid prints.

Thickness measurements

The lesion was delineated from the A-scan trace and its ultrasound propagation time measured. This time was subsequently converted into the ultrasound thickness measurement by multiplication by the velocity of sound in skin. This has been taken to be 1580 m/s in this study.[6]

The tumour thickness was also assessed histologically from the excised specimens. Between two and four sections from the centre of each lesion were measured, corresponding to the area from which the A-scan was measured. Thickness measurements were made from each section using side-arm projection of the microscope image and a digitizing tablet linked to a Hewlett Packard 85 computer. The mean of five thickness measurements from each section was used to give a mean histological depth from the central part of the lesion.

Diagnostic features of the A-scan trace

Amplitude. The difference in echo amplitude between the tumour and the tissue above and below allows the identification, localization and measurement of abnormal tissue. Four measurements were taken of the amplitude of the echoes at equal intervals in the area of skin of interest, and their mean value computed. This was defined as the amplitude value.

Density. The number of echoes per unit time (density) arising from the tumour relates to its structure; a tumour with an entirely homogeneous structure would not give any echoes. The total number of echoes from within the tumour (or from the whole of the normal skin trace) was counted. This value was then divided by the tumour or skin trace time, giving a density figure in echoes per microsecond. A simple conversion of the time to a tissue distance gives a figure in echoes per millimetre.

Regularity. Tumours with an irregular arrangement of a single type of tissue would show irregular spacing of echoes (with even amplitude). Spacing between echoes represents spacing of tissue components (e.g. density of collagen fibres). A figure for regularity was arrived at by counting the number of changes in height and the number of changes in spacing of serial pairs of echoes (e.g. measurements between echo numbers 1 and 2, 2 and 3, 3 and 4, etc.) within the tumour region. This figure was then divided by tumour or whole skin trace time, and a regularity figure obtained in 'changes per microsecond.'

Attenuation. An approximate idea of the attenuative property of the tumour tissue can be obtained by noting the amplitude of echoes arising from any normal dermis beneath the tumour. In this study a simple measurement of peak amplitude of echoes from beneath the tumour was taken and used to indicate the attenuation introduced by the tumour tissue.

RESULTS

The results of the comparison between A-scan and histological thickness measurements of all the tumours, using an ultrasound velocity in skin of 1580 m/s are shown in Figure 1. As

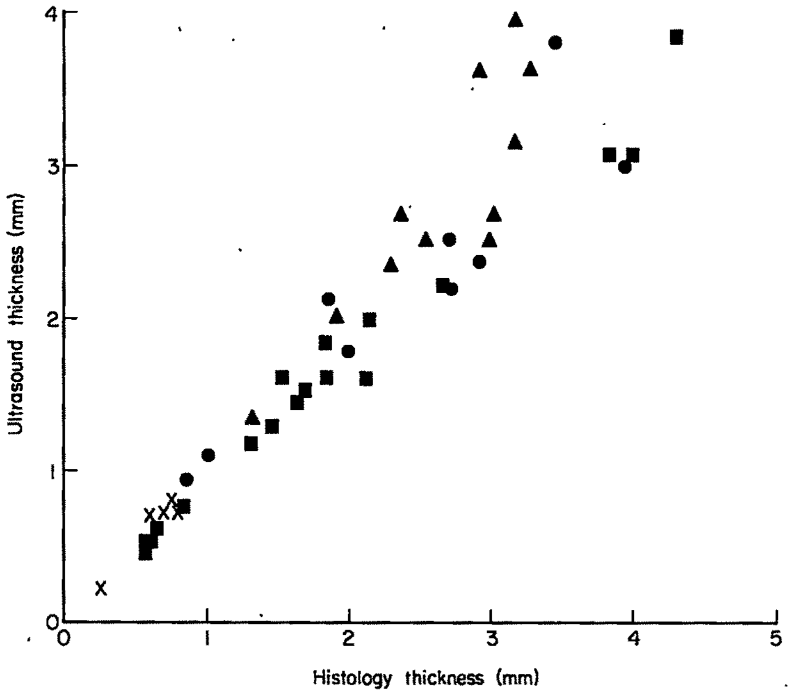

FIGURE 1. Comparison between histological and ultrasound thickness measurements of small skin tumours. Linear regression model gives slope of 0·94, correlation coefficient of 0·96. ■, BCC; ●, Moles; ▲, Fibrosis; X, IEE.

TABLE 1. Mean ± standard deviations of measured features for tumour and adjacent normal skin control sites

	Amplitude		Density		Regularity		Attenuation		Thickness	
BCC	0·15±0·06	*	7·2±2·5	*	2·5±1·2	*	0·6±0·4	NS	1·80±1·10	*
Control site	0·63±0·09		11·0±2·2		8·9±1·15		0·7±0·3		2·16±0·44	
Mole	0·12±0·06	*	5·2±1·7	*	1·8±0·7	*	0·32±0·25	*	2·60±1·70	*
Control site	0·64±0·25		10·1±2·2		5·3±0·7		0·84±0·96		1·83±0·52	
Fibrosis	0·24±0·09	*	10·4±1·7	NS	5·1±0·8	*	0·6±0·5	*	2·60±1·0	*
Control site	0·89±0·32		11·8±2·2		6·1±1·1		1·14±0·75		1·80±0·60	
Bowens	0·94±0·33	*	10·3±0·9	*	3·0±1·5	*	0·50±0·36	NS	0·56±0·08	*
Control site	1·15±0·36		11·4±0·6		5·6±0·8		0·76±0·36		2·21±0·87	

* = Significant difference, $P<0·05$ (Mann–Whitney *U*-test), NS = Not significant.

expected, the two methods showed a good correlation, but the ultrasonically derived thicknesses were consistently lower than the histologically measured value.

The mean and standard deviations of the A-scan features measurements are presented in Table 1. Since the tumours were of differing sizes and maturity, and were from differing sites, mean values of their features do not allow clear distinction between tumour types, although in most measurements significant differences between lesional and normal sites are apparent from Table 1. In order to account for variations in site and in generalized skin changes (e.g. due to solar elastosis) values of the four features measured from a lesion were normalized with respect to the adjacent uninvolved skin. Each relative value is computed by dividing the value from unaffected adjacent skin by the value from the lesion, so that site and generalized skin changes are accounted for. To determine how the chosen features distinguished between tumours, a Mann–Whitney *U*-test was performed, and the results are presented in Table 2.

The relative amplitude feature separated all tumours except BCC from the fibrosis group.

TABLE 2. Discrimination between tumours by A-scan ultrasound features. 'Relative' features are compared to normal adjacent site

	Relative amplitude	Relative density	Relative regularity	Relative 'attenuation'	Absolute thickness
BCC vs. Mole	*	NS	**	*	*
BCC vs. Fibrosis	NS	***	***	NS	**
BCC vs. IEE	***	**	NS	NS	***
Mole vs. Fibrosis	**	***	***	NS	NS
Mole vs. IEE	***	**	**	**	**
Fibrosis vs. IEE	***	NS	*	**	**

Mann–Whitney *U*-test: NS = Not significant. * $P=0·05$, ** $P=0·02$, *** $P=0·002$, (2-tail probabilities).

This could be due to the fact that some nodular BCC tumours exhibited larger areas of normal tissue between tumour cell foci. The relative density separated all types except BCC from moles and fibrosis group from IEE tumours. BCC and moles are mechanically quite similar, being extensive cellular tumours, so their echo spacing (density) relative to normal skin will be similar. The fibrosis group was not separable from the IEE group, probably because of the few numbers (five) in the IEE group.

The relative regularity (representing the homogeneity of the tumour) provided good discrimination, failing only to separate BCC from IEE. Again, the low number of IEE studied precludes any firm conclusions from this observation. Relative attenuation is very dependant on tumour thickness, so separation of tumour types is best between generally thick tumours and generally thin tumours (i.e. between IEE and mole or fibrosis). The absolute thickness of the

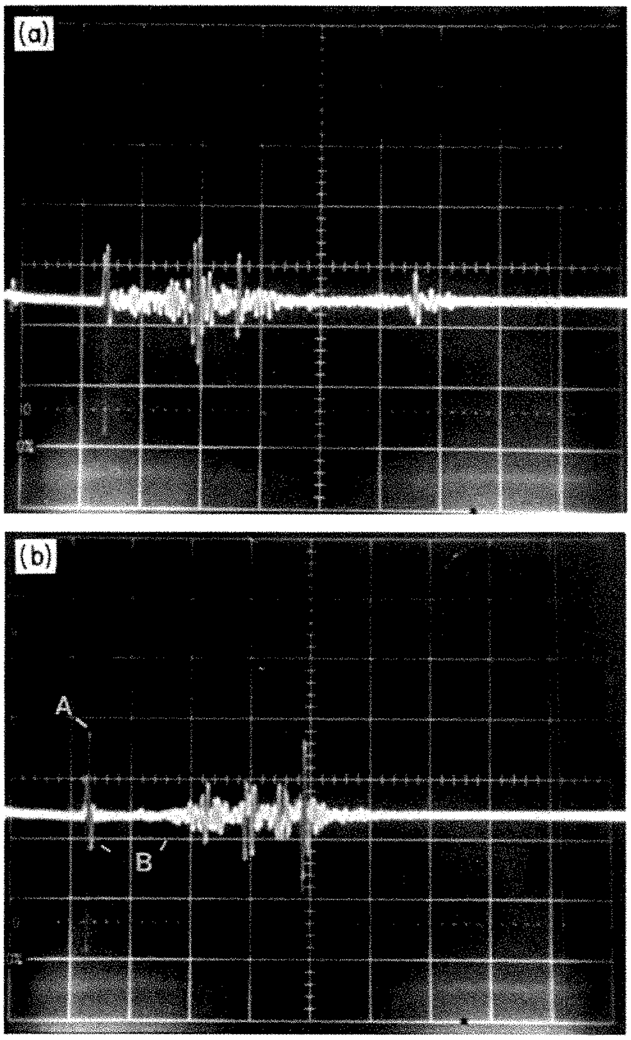

FIGURE 2. (a) A-scan from normal skin, side of nose. (b) A-scan from adjacent BCC. A, skin-water interface, B, tumour mass. Vertical scale, 100 mV per large division. Horizontal scale, 1 μs per large division.

tumours is of course dependant on the age and activity of the tumour. However, in the group of tumours studied only comparisons between fibrosis and moles failed to reach significance. This is because, as shown in Table 1, the group of moles and the fibrosis group had the same mean thickness.

Figure 2 illustrates a superficial BCC. The low amplitude region 'B' in the tumour scan is immediately obvious. The single echo 'A' from the skin surface leads directly into the tumour, showing the absence of a thick stratum corneum or crust, and illustrating that the tumour arises from the epidermis. Some small echoes are present within the tumour zone, suggesting a non-homogeneous tissue distribution. The echoes beneath the tumour indicate a thinner dermal layer than the adjacent normal dermis, showing that this tumour is invading the dermis.

Figure 3 shows the A-scan from a scar. The difference between the adjacent normal skin and

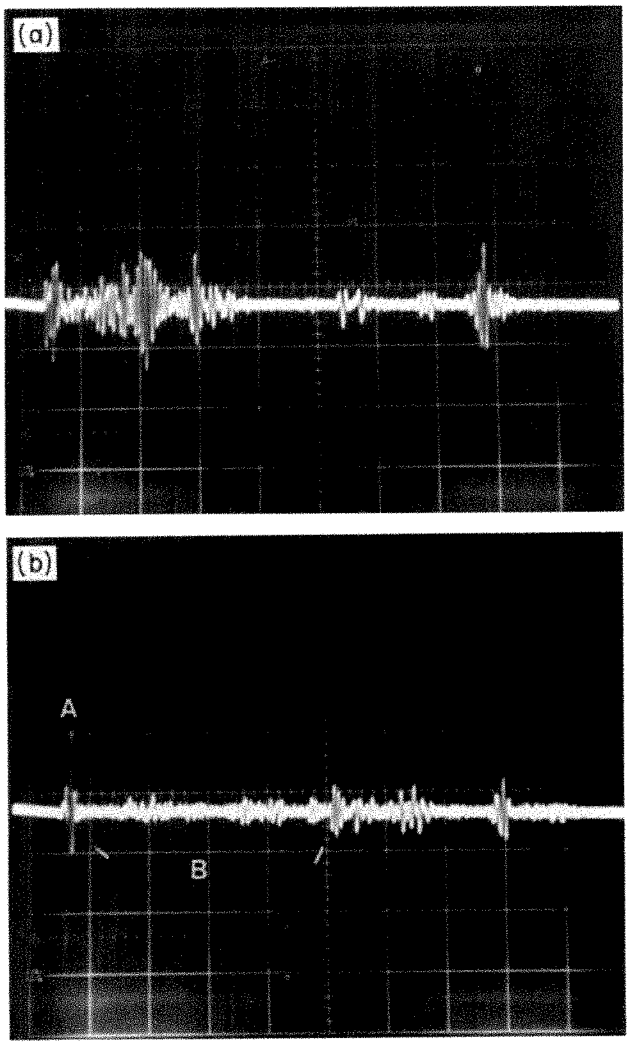

FIGURE 3. (a) A-scan from normal skin, face. (b) A-scan from adjacent scar tissue. A, skin–water interface; B, scar tissue. Vertical scale, 50 mV per large division. Horizontal scale, 1 μs per large division.

the affected area is clear. The scar tissue, 'B', is more echogenic than either the BCC or the other tumours studied because it is composed of randomly orientated fibrous components.

For most cases the interface between lesional and underlying dermal tissues is not immediately obvious from the static Polaroid photograph. However, during the A-scan examination a variation of the pressure of the probe on the skin shows a difference in compressibility between lesional and dermal structures which clearly indicates their interface. This is noted and marked on the Polaroid border at the time of examination.

DISCUSSION

Tumour tissue is measured in the A-scan method by the transit time of the acoustic pulse. This transit time is then transformed into thickness by the speed of sound in the tissues. For normal skin this speed is about 1580–1640 m/s[6] but for cellular tissue, e.g. a BCC, this may drop to about 1540 m/s.[7] This change is too small however to have much effect on our measurements, since other sources of error are potentially much greater. The most significant of these will be, (i) the exact correspondence between the sampling position *in vivo* and on the histological specimen; this we have minimized by ensuring that both measurements are taken from the same area of tissue and that the histological measurement is a mean, which should minimize the effects of uneven tumour margins, and (ii) the effects of preparation of tissue. The excellent correlation found between methods shows that our interpretation of the tumour echoes corresponds to the histological interpretation. Differences in absolute values between methods is simply due to the increase in thickness occurring when tissue is removed from *in vivo* tension and undergoes histological preparation,[2] and it may be that the ultrasound measurement is the more useful.

In distinguishing lesional from normal tissue A-scan traces, Table 1 shows that the amplitude and the regularity (changes in echo amplitude and/or spacing per unit depth of tissue) are consistently significantly different. This is because all of the tumours have different mechanical composition and structure from normal dermal collagenous tissue.

The density of echoes (number of echoes per unit depth of tissue) is significantly different between the three cellular tumours (BCC, mole and IEE) and normal, but not between the fibrotic tumours (scars, dermatofibromas) and normal collagenous dermis.

The attenuation figure (amplitude of echoes beneath the tissue of interest) is highly dependent on the thickness of the lesional tissue. For lesions thinner than normal dermis (BCC and IEE) no significant difference in the attenuation figure was observed. For the thicker lesions, the attenuation figures were low enough to be significantly different from the surrounding normal tissue. In all lesions, the abnormal tissue was either significantly less thick (BCC, IEE) or significantly thicker (fibrosis, mole) than normal dermis.

Table 2 shows how well our features distinguish between different tumours. The two tumours which were most consistently different were moles and IEE. This was probably due to the fact that the thick moles produce the lowest echoes beneath their tissue mass, whereas the thinner IEE leave a relatively normal dermis beneath. Clearly more examples of these tumours are required before authoritative statements can be made regarding the IEE tumours.

The best single distinguishing feature between tumours was the amplitude, and the IEE mean amplitude was significantly nearer to normal than the other lesion amplitudes (Table 1). Highly significant differences in relative amplitude between IEE and other lesions were found (Table 2).

A-scan traces from BCC tumours were distinguished (i) from IEE A-scans by the presence of a thicker lesional area containing lower amplitude echoes from the BCC, (ii) from fibrotic tissue by the presence of a thinner lesional area exhibiting less dense and less regular echoes from the BCC, (iii) from moles by the presence of higher amplitude, less regular lesional echoes and by the presence of higher echoes from beneath the BCC.

The quantification of the A-scans from Polaroid photographs is laborious. However, with the advent of cheap and fast digitizing oscilloscopes coupled to cheap and powerful microcomputers the automatic computation of the A-scan features described here is feasible. This would enable 'instant' quantitative A-scan feature assessment, bringing ultrasound-derived tissue characterization[8] within the realms of possibility for routine dermatological practice.

This work on quantifying A-scan features is a necessary preliminary to attempting similar studies with B-scans. We have shown that simple quantification of echoes allows differentiation between the tumours and future studies will extend this method into different skin conditions.

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The localization of the bullous pemphigoid and cicatricial pemphigoid antigens: direct and indirect immunofluorescence of suction blisters

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SUMMARY

The location of *in vivo* bound immunoreactants was studied in 37 patients with subepidermal blistering diseases by direct immunofluorescence (IMF) on suction blisters taken from uninvolved forearm skin. The patients studied included 18 with bullous pemphigoid (BP), nine with cicatricial pemphigoid (CP), three with acquired epidermolysis bullosa (EBA) and 7 hybrid cases. The patterns of IMF in the suction blisters were: BP, epidermal 1, dermal 1, combined 4, negative 12; CP, epidermal 1, dermal 2, negative 6; EBA, dermal 2, negative 1; and 'hybrid' patients, epidermal 3, negative 4. The different patterns of suction blister staining could not be correlated with the clinical features of the patients in respect of mucous membrane involvement, scars or milia or a history of skin fragility. Both BP and CP are probably heterogeneous in respect of their antigen specificity, and the two diseases cannot reliably be distinguished by the patterns of direct IMF on suction blisters. In addition, some individual patients with BP have more than one target antigen as indicated by a combined pattern of suction blister fluorescence. The lack of correlation between the pattern of suction blister fluorescence and the clinical features suggests that factors other than antigen specificity determine the clinical expression of subepidermal blistering diseases.

Bullous pemphigoid (BP) and cicatricial pemphigoid (CP) have identical histological, immunopathological,[1–5] electron microscopic[6,7] and immunoelectron microscopic findings.[8–13] However, in spite of these similarities, CP has been regarded as a clinical entity distinct from BP, because of its predilection for the mucous membranes and tendency to form scars.

Direct and indirect immuno-electron microscopy indicate that the target antigens in both BP and CP are localized within the lamina lucida.[8–13] However, the ultrastructural localization of the BP and CP antigens within the lamina lucida may differ. Using suction blisters or chemically split skin, which are techniques known to separate the epidermis from the dermis through the

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lamina lucida, the binding of immunoreactants in BP occurs principally along the epidermal side of the split[14] whereas in CP immunoreactants have been detected along its dermal aspect.[13,15] It has been hypothesized that the CP antigen is distinct from the BP antigen and occupies a position lower down in the lamina lucida than the BP antigen.

We have studied 18 patients with BP and nine with CP to investigate whether BP and CP could be distinguished by the location of *in vivo* bound immunoreactants in suction blisters taken from uninvolved forearm skin. The findings were compared with those obtained in three patients with acquired epidermolysis bullosa (EBA), in which the target antigen is known to be type VII collagen in the upper dermis, and also with seven 'hybrid' patients, who had BP-like diseases but with clinical features overlapping with CP or EBA. The direct immunofluorescence (IMF) findings in suction blisters were compared to the indirect IMF findings using patients' sera on split normal skin substrates. An attempt was made to relate the location of the antigens identified by IMF to the clinical expression of the diseases.

METHODS

Patients

The clinical details of the patients are shown in Table 1.

Bullous pemphigoid. BP had been diagnosed in 18 patients (12 males, 6 females; age range 49–83 years, mean age 68 years; disease duration 1 month–12 years, mean 4 years 1 month). The criteria for inclusion in the BP group were non-scarring subepidermal blistering and with positive direct IMF in the basement membrane zone (BMZ) of uninvolved skin. Thirteen of the 18 patients had mucous membrane lesions at no more than two mucosal sites. Milia were noted in seven patients. The presence of milia was not regarded as an exclusion from the BP group, as we have frequently observed transient milia in the resolving skin lesions of patients who in all other respects fulfil the criteria of typical bullous pemphigoid and have not developed scars.

BP/CP overlap patients. Five patients had blistering disorders with positive IMF with clinical features intermediate between classical BP and CP. All had predominantly mucous membrane pemphigoid, and four developed scars but only after a prolonged period of non-scarring disease.

Cicatricial pemphigoid. Nine patients (3 males and 6 females; age range 50–72 years, mean age 57 years; disease duration 1 year–21 years 8 months, mean 8 years 1 month) were diagnosed as having CP, on the basis of subepidermal blisters of mucous membranes, or skin and mucous

TABLE 1. Summary of the clinical features of patients studied

Diagnosis	Number of patients	Clinical features				
		Skin	Mucosae	Scars	Milia	Fragility
BP	18	18	12	0	7	0
BP/CP overlap	5	5	5	4	2	0
CP	9	7	9	8	1	0
BP/EBA overlap	2	2	2	0	0	2
EBA	3	3	2	3	3	3

membranes together, with scarring. In two patients the disease was confined to the mucous membranes, while in seven mucosal and skin lesions occurred together, accompanied by scarring. In only one of these patients were milia observed, possibly because the skin lesions were sporadic and therefore freshly resolving lesions were infrequently observed. Six patients had positive direct IMF of skin or mucous membrane at the time of diagnosis and one was consistently negative.

Pemphigoid/EBA overlap. Two patients (1 male, 1 female; aged 57 and 75 years; disease duration 2 years 3 months and 2 years 2 months) had a history of skin fragility, with otherwise typical IMF-positive BP.

EBA. Three patients (1 male, 2 female; age range 42–55 years, mean age 49 years) had 'classical EBA' with erosions and blisters occurring both in response to minor trauma and spontaneously. Lesions predominated on the dorsa of hands and feet and over the elbows and knees, and healed with milia and scarring. Two also had lesions of the oral mucosa.

Skin separation methods

Mechanical separation. Suction blisters were raised on the volar aspect of the patients' forearm on uninvolved skin, as described by Kaplan *et al.*[16] Briefly, a glass suction cup with five holes of 5 mm diameter was attached to the patient's forearm and connected to an Eschmann CP50 suction pump operating at a negative pressure of 200 mmHg. The mean time to the development of two blisters was noted in each patient. One blister from each patient was biopsied intact by a 3 mm punch biopsy under local anaesthesia. A second 3 mm biopsy of adjacent unblistered skin was also taken and both specimens were embedded in OCT compound and immediately snap frozen in liquid nitrogen for IMF studies. In eight patients, a second suction blister was also biopsied and fixed in 4% glutaraldehyde for electron microscopy in order to confirm the ultrastructural location of the split.

Chemical separation. Normal skin from a healthy volunteer was separated by incubation in freshly prepared 1·0 M NaCl solution at 4°C or at room temperature. The epidermis was then gently separated from the dermis using forceps. The specimen was embedded in OCT compound and immediately snap frozen in liquid nitrogen for IMF studies.

Direct immunofluorescence

Cryostat sections, 6μm-thick of unfixed suction blisters were cut and used for direct immunofluorescence testing using fluoroscein labelled rabbit anti-human immunoglobulin (Hoechst) against IgG, IgA, IgM, C3, C4 and C_{1q}.

Indirect immunofluorescence

Indirect IMF was performed using 6 μm cryostat sections of the following substrates: intact skin, suction blister-split skin and NaCl-split skin, taken from healthy donors. A reference BP serum (gift from Dr J. Stanley) which gave roof only fluorescence on split skin in preliminary experiments was used as a positive control for roof fluorescence, and an immunoblotted EBA serum (gift from Dr M.M.Black) was used as a positive control for floor fluorescence in the split skin studies. Normal human serum was used as a negative control.

Antigen mapping of the level of the split

The level of the split was examined in eight suction blisters (two BP, three CP and three BP/CP overlap) by indirect IMF using the following reference sera: bullous pemphigoid (gift from Dr J.Stanley), anti-laminin (Bethesda Research Laboratories), anti-type IV collagen, EBA serum immunoblotted against type VII collagen (undiluted) (gift from Dr M.M.Black). Three of the blisters examined by indirect IMF contained *in vivo* bound immunoreactants in the blister roof, but the remainder were negative on direct IMF.

Electron microscopy

In eight (seven BP, one CP) patients a second suction blister was biopsied and fixed in 4% glutaraldehyde for electron microscopy. The tissue blocks were post-fixed in osmium tetroxide prior to routine processing to EMIX resin. Thick sections were stained with toluidine blue and areas including the blister and its junction with intact skin were selected for ultra-thin sectioning. The ultra-thin sections were stained with lead citrate and uranyl acetate and examined by electron microscopy.

RESULTS

Blistering time

The mean time for the formation of two blisters was similar in all groups of patients (range 20–120 min; mean 48 min).

Confirmation of the site of suction blister formation

Electron microscopy of suction blisters from six patients with BP and one with CP showed the split occurring in all specimens in the region of the lamina lucida with the lamina densa intact and always lying on the dermal side. Cellular debris and melanocytes were sometimes adherent to the lamina densa, but this only occurred at the edge of the blister and never away from this edge zone. The epithelial cells of the roof of the blister included an intact plasma membrane. Antigen mapping by indirect IMF of suction blisters from two BP, three CP and three BP/CP overlap patients gave the same result in all cases. An epidermal pattern of IMF was obtained using a standard BP serum, whereas a dermal pattern of fluorescence was obtained when anti-laminin, anti-type IV collagen or an EBA serum were used.

Direct immunofluorescence studies

The results are shown in Table 2.

Bullous pemphigoid. Five patients had positive linear direct IMF with IgG and/or C3 in the BMZ of uninvolved intact skin; one patient had linear deposition of IgM. Of these six patients the suction blister IMF was negative in two and positive in four. A further two patients had positive IMF within the suction blister despite negative findings in intact skin. The patterns of suction blister staining obtained were: epidermal only one; dermal only one (Fig. 1); combined four (Fig. 2).

BP/CP overlap patients. The intact skin was positive on direct IMF in one of the five BP/CP overlap patients and this patient gave an epidermal pattern of staining in the suction blister.

TABLE 2. The distribution of positive direct IMF in suction blisters

	No. with + direct IMF			
		Suction blister		
Diagnosis	Intact skin	Epidermal only	Dermal only	Combined
BP	6	1	1	4
BP/CP overlap	1	2	0	0
CP	2	1	2	0
EBA	2	0	2	0
BP/EBA overlap	1	1	0	0

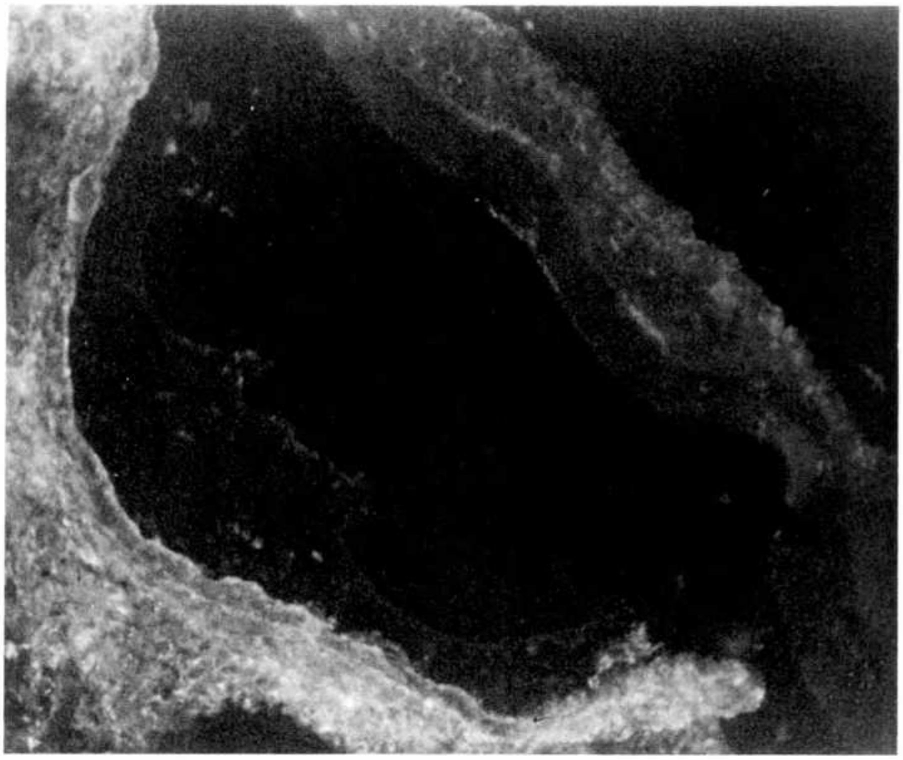

FIGURE 1. Direct immunofluorescence of a suction blister from a bullous pemphigoid patient showing a linear band of IgG along the dermal side of the split.

Cicatricial pemphigoid. Two of the nine CP patients had positive direct IMF in both intact skin and suction blister, and a further patient whose IMF was negative in intact skin, was none the less positive within the suction blister. The patterns of suction blister IMF were epidermal in one and dermal in two.

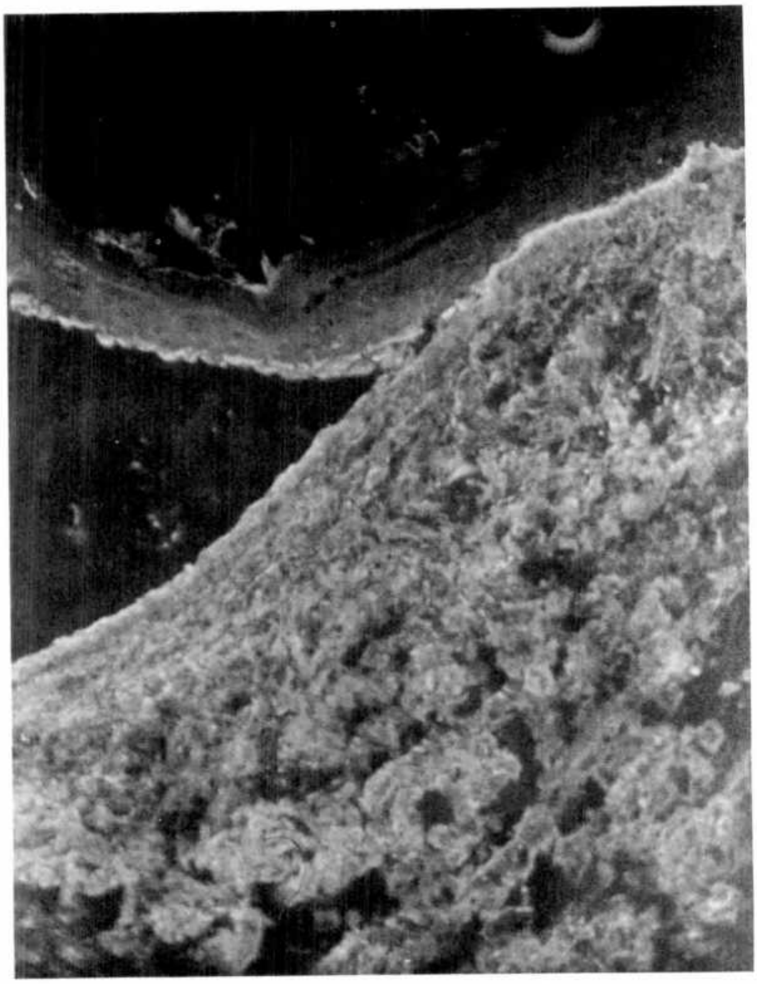

FIGURE 2. Direct immunofluorescence of a suction blister from a bullous pemphigoid patient showing fluorescence along both epidermal and dermal sides of the split.

Bullous pemphigoid/EBA overlap. One patient had positive direct IMF in both intact skin and on the epidermal aspect of the suction blister roof. An additional patient whose intact skin was negative also gave an epidermal pattern in the suction blister.

EBA. Two EBA patients had positive direct IMF in the intact skin specimen and both gave a floor only pattern of staining in the suction blister.

Correlation of suction blister direct IMF with clinical features

The patterns of suction blister fluorescence were compared with the presence or absence of key clinical features, namely mucosal involvement, scarring, milia and a history of skin fragility. The results are shown in Table 3. No clear relationship was apparent between the antigens detected and the clinical expression of the diseases. Fluorescence exclusively on the dermal

TABLE 3. Analysis of suction blister direct IMF and clinical features

Clinical feature	No. of patients ($n=37$)	Pattern of direct IMF on suction blister: Negative ($n=23$)	Epidermal ($n=5$)	Combined ($n=4$)	Floor ($n=5$)
Mucosal involvement	30	19	4	2	5
No mucosal involvement	7	4	1	2	0
Scars	15	9	2	0	4
No scars	22	14	3	4	1
Milia	12	7	1	2	2
No milia	25	17	4	2	2
Fragility	5	1	2	0	2
No fragility	32	22	3	4	3

aspect of the suction blister was not observed in patients without mucosal involvement, though combined epidermal and dermal fluorescence was observed in this group.

Indirect immunofluorescence studies

There was good agreement between the results obtained on NaCl split skin and on the suction blister-derived substrate, with the exception of one serum which was positive (epidermal) on NaCl split skin but negative in the suction blister, and three BP sera which produced small quantities of fluorescence along the dermal aspect of the suction blister, though not the chemically split skin.

Indirect IMF was positive in 10/18 BP patients using intact skin as substrate. Using NaCl-split and suction blister split skin, positive results were obtained in 11/18 and 10/18 respectively. All of the positive BP sera gave an epidermal pattern in the split skin substrates, with the exception of three which produced weak and patchy fluorescence along the blister floor, as well as strong fluorescence in the roof. In the BP/CP overlap group, all four sera were positive on intact skin and all gave epidermal fluorescence in both the suction blister and the NaCl-split substrates. Only one CP serum was positive using intact skin and this serum bound to the roof of both the split skin substrates. An additional CP serum bound to the roof of the suction blister but was negative in intact skin and in the NaCl-split skin. Both of the two pemphigoid/EBA overlap sera were positive on intact skin and along the roof of both the suction blister and the NaCl-split substrates. None of the EBA sera gave positive fluorescence with any substrate.

Comparison of direct and indirect IMF findings in split skin

The results are shown in Table 4. In 12 patients both direct and indirect IMF was positive in split skin, permitting comparison of the patterns of fluorescence. Antigens along the dermal aspect of suction blisters, detected by direct IMF, were undetectable on indirect IMF except in one case which gave small quantities of fluorescence along the floor of the suction blister split skin, though not the chemically split skin.

TABLE 4. Comparison of direct and indirect IMF finding in intact and split skin

Direct immunofluorescence				Indirect immunofluorescence				
		Suction blister			NaCl split skin		Suction blister	
Diagnosis	Intact skin	Epidermal	Dermal	Intact skin	Epidermal	Dermal	Epidermal	Dermal
BP	C3	—	—	+	+	–	+	–
	—	IgG	—	+	+	–	+	–
	IgG C3	IgG C3	IgG C3	+	+	–	+	–
	IgG C3	IgG C3	IgG C3	+	+	–	+	–
	IgG C3	IgG	IgG C3	–	–	–	–	–
	—	C3	C3	–	–	–	–	–
	IgG	–	IgG	+	trace	–	+	trace
BP/CP overlap	IgG C3	IgG C3	–	+	+	–	+	–
CP	–	–	IgG	–	–	–	–	–
	IgG	–	IgG	+	+	–	+	–
BP/EBA overlap	–	IgG	–	+	+	–	+	–
	IgG C3	C3	–	+	+	–	+	trace
EBA	IgG IgA C3	–	IgG IgA	–	–	–	–	–

DISCUSSION

The currently available criteria for the diagnosis of BP and CP are clinical, as the two diseases are indistinguishable by immunopathology, electron microscopy or immunoelectron microscopy.[1–13] In a previous study we demonstrated the lack of specificity of these clinical criteria, and emphasized the degree of clinical and immunopathological overlap between the two diseases.[17] In the present study we have attempted to define BP and CP by strict clinical criteria in an attempt to ensure maximum homogeneity between patients in each group. Patients with overlapping clinical features were assigned to separate 'hybrid' groups.

The suction blister technique was used to separate the lamina lucida into epidermal and dermal compartments, so that antigens located in these compartments could be separately identified. The validity of this model was confirmed by antigenic mapping of the suction blisters using reference sera, which showed that as far as the major known antigenic determinants of the BMZ are concerned there was a 'clean break' at the molecular level into epidermal and dermal compartments.

Although two EBA patients had floor only fluorescence in their suction blisters, consistent with the upper dermal location of the EBA antigen, more variable results were obtained among the BP and CP patients. The BP patients yielded three distinct patterns of suction blister fluorescence; epidermal, dermal and combined. This implies that patients with BP are heterogeneous with respect to target antigen specificity, with some patients having antibodies directed against antigen(s) in the epidermal portion of the lamina lucida while others also have target antigen(s) lower down, in the dermal compartment of the lamina lucida. Furthermore, the finding of combined roof and floor patterns suggests that individual BP patients possess antibodies of more than one specificity.

Among the CP patients, two had immunoreactants located exclusively on the dermal aspect of the suction blister, identical to that seen in the EBA patients, whereas one had epidermal binding only. This suggests that in CP, as in BP, there is heterogeneity of target antigen specificity. An epidermal pattern of suction blister fluorescence has been reported before in a single CP patient[15] so this pattern appears to be a genuine variant of CP in terms of target antigen specificity. The small numbers of CP patients studied preclude any estimate of its frequency.

The pattern of staining obtained in the hybrid groups was of interest as these patients demonstrated clinical overlap between BP and CP or BP and EBA. Two of the BP/CP overlap patients and one in the BP/EBA overlap group had epidermal fluorescence, while no patient gave a dermal or combined pattern. One of these 'roof-only' hybrid patients had both non-scarring skin involvement, and persistent desquamative gingivitis. The latter clinical picture has been associated in some cases with cicatricial pemphigoid,[18] and in others with a mechanobullous disorder.[19] The other two hybrid patients were so designated because of a scarring nail dystrophy in one and a history of skin fragility in the other. Despite these CP- and EBA-like clinical features none of the hybrid patients had any immunoreactants bound on the dermal aspect of their suction blisters. Furthermore, when all the patients were analysed together, we found a lack of correlation between certain distinctive clinical features such as mucous membrane involvement, milia, scars and skin fragility and the pattern of suction blister direct IMF (see Table 3), suggesting that the clinical expression of the subepidermal bullous diseases is not primarily determined by the specificity of the target antigen.

The nature of the antigenic determinants detected in the suction blisters of BP patients can only be inferred as parallel immunoblotting studies of these sera have not yet been performed. Previous immunoprecipitation[20] and immunoblotting[21] studies of BP sera have identified a 220–240 kDa polypeptide, which is recognized by most sera so far tested and appears to be a major BP antigen. However, bands of molecular weight 180–200 kDa, as well as bands of lower molecular weight, have also been recognized by BP sera on immunoblots of epidermal extracts,[22–24] and one or more of these antigenic determinants could be responsible for the epidermal pattern of fluorescence in split skin studies. Still less is known of the nature of dermis-associated antigenic determinants in BP and there have been no reports of immunoblotting of BP sera against extracts of the dermal component of split skin.

There have been few immunoblotting studies of CP sera, as the circulating antibody is frequently undetectable or present only in a low titre. However, a preliminary report suggests that some CP sera identify 120 kDa and 240 kDa polypeptides in immunoblots against extracts of heat separated epidermis.[24] This result suggests firstly, that at least some of the target antigens in CP are 'epidermis-associated', a finding which could be consistent with an epidermal pattern of suction blister IMF. Secondly, it points to there being a common 240 kDa antigen, shared by BP and CP, as well as a unique 120 kDa CP antigen. It remains to be seen whether this finding is confirmed by immunoblotting of larger numbers of CP sera. As with BP, there is no evidence concerning the nature of the dermal-associated CP antigens.

Indirect IMF on split skin gave an epidermal pattern of fluorescence in most BP and CP patients in agreement with previous reports.[25,26] Both dermal and combined patterns of indirect fluorescence have previously been obtained using BP and CP sera on split skin substrates,[25,26] though only in a minority of cases. Only three of our BP sera, and none of the CP, EBA or hybrid sera produced a combined pattern. Hence the indirect IMF findings did not correlate in all cases with the pattern of direct IMF in suction blisters. The reason for this lack of concordance is unknown. Possibly some of the antibodies concerned are of low titre and/or low affinity and are therefore not consistently detected. This appears to be particularly true of the antibodies binding to the antigen(s) in the dermal aspect of suction blisters; of nine patients with *in vivo* binding to floor antigens, in only one was a corresponding anti-floor antibody detected and even in this one the fluorescence along the blister floor was faint and patchy.

We conclude that even when BP and CP are strictly defined, both are probably heterogeneous in respect of their antigen specificity, and the two diseases cannot reliably be distinguished by the patterns of direct or indirect IMF on split skin. In addition, at least among the BP patients, there is also heterogeneity of antigen specificity within some individual patients, as indicated by a combined pattern of suction blister direct IMF. The location of the target antigen in split skin does not correlate with the clinical features, suggesting that factors other than antigen specificity determine the clinical expression of subepidermal blistering diseases. Finally these studies emphasize that identification of the antigen(s) in the dermal compartment of the lamina lucida will be necessary to clarify both BP and CP and the relationship of these clinical entities to one another.

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Arachidonic acid transformation is not stimulated in delayed pressure urticaria

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SUMMARY

Little is known about the molecular mechanisms or inflammatory mediators involved in delayed pressure urticaria (DPU). Pressure sufficient to provoke lesions was applied to the back of six patients with DPU. The levels of products of arachidonic acid transformation in skin exudate from the pressure challenged skin were estimated immediately after pressure was removed and 6 h later when lesions were present. These were compared to levels estimated in a similar way from unchallenged skin in these patients. Levels of leukotriene $C_4/D_4/E_4$, prostaglandin E_2, 12-hydroxyeicosatetraenoic acid and leukotriene B_4 were not raised in lesional skin. Our results suggest that arachidonic acid metabolism is not stimulated in DPU.

Delayed pressure urticaria (DPU) presents as erythematous swellings which occur from 30 min to 9 h after pressure is applied to the skin. The swellings are itchy and painful and sometimes persist longer than 24 h.[1] Histopathology of lesions which are present for less than 5 h show aggregations of neutrophil and eosinophil leucocytes in the dermis which may extend deeply into the fat. Lesions which are present longer than 5 h show aggregates of lymphocytes and eosinophils.[2] Little is known about the mechanism or mediators of inflammation involved in the initiation of the lesions or the mechanisms which cause the lesions to persist. The level of histamine was found to be raised in one patient using a suction blister technique.[3] However, the unresponsiveness of the condition to antihistamine treatment[4] makes it unlikely that histamine is of major importance in the pathogenesis of DPU. Since the reaction is sustained, mediators related to the cellular infiltrate are likely to be involved. Many eicosanoids have potent inflammatory actions in human skin. Leukotriene B_4 (LTB_4) is a potent chemoattractant both *in vitro* and *in vivo* and when injected into the skin it causes a prolonged erythematous induration in which neutrophils and eosinophils are found.[5] The pro-inflammatory properties of 12-hydroxyeicosatetraenoic acid (12-HETE) are similar to LTB4 but LTB_4 is less potent.[6]

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Mediators generated by the infiltrating cells, including LTB_4 from neutrophils and $LTC_4/D_4/E_4$ from eosinophils, may enhance and extend the inflammatory reaction. Prostaglandin E_2 (PGE_2) is also a potent vasodilator which is released during many inflammatory reactions and may act synergistically with the other inflammatory mediators.[7] In this study we investigated the release of eicosanoids in the skin of patients with DPU.

METHODS

Ethical approval for this study was obtained from the St Thomas's Hospital Ethics Committee and all patients gave written informed consent. Six patients (three male), aged between 30 and 51, with delayed pressure urticaria were recruited. Each gave a history of swelling developing some hours after application of pressure to the skin, and each developed papular responses 6 h after application of $9{\cdot}75 \times 10^5$ Pa to the skin of the back using a calibrated dermographometer.[8] All patients suffered from concurrent chronic urticaria but no other dermatological disorder was present. No drug treatment had been taken for either condition for at least 1 week prior to the study.

Pressure was applied to the skin of the back using an apparatus based on that of Illig and Kunick.[9] Stainless steel rods, 1·5 cm in diameter and of known weight were placed perpendicularly on the surface of the back lateral to the thoracic spine (Fig. 1). Pressure was varied by adding additional weights to the rods. As the patients' responses varied and the swelling produced is dependent both on the pressure applied and the duration of application of pressure,[10] all patients were pretested to determine the pressure/time necessary to produce approximately equal swellings of at least 2 cm diameter at 6 h. Weights required ranged between 2·29 kg and 4·79 kg and duration of application varied between 12 and 15 min. Pressure challenge was applied to four sites on each patient.

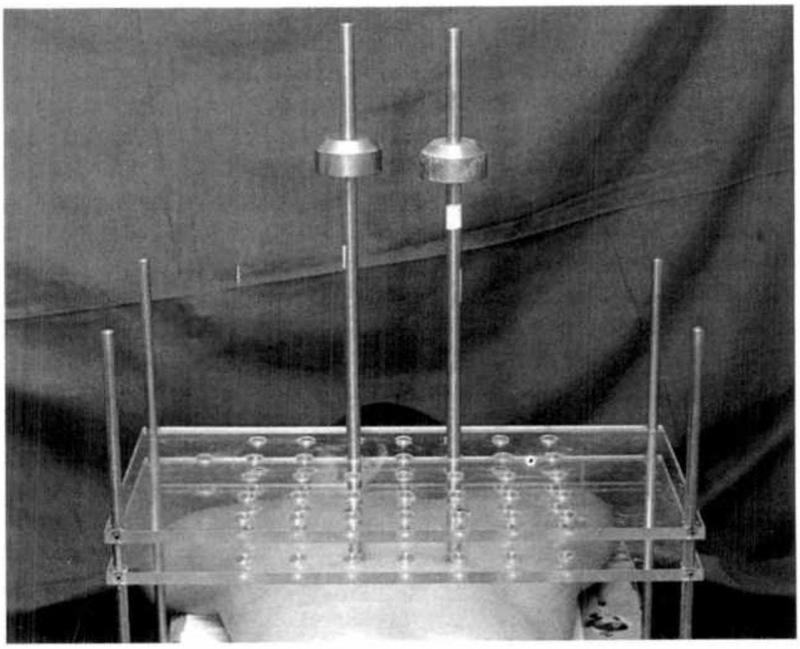

FIGURE 1. Pressure instrument with weighted rods resting on patients back.

Two skin abrasions, 1·9 cm diameter, were prepared immediately after pressure was removed (time zero) and at 6 h when erythematous palpable swellings had developed. In addition, two abrasions were prepared on control unchallenged sites.

Skin exudates were collected in 0·8 ml phosphate-buffered saline using the chamber technique.[11] PGE_2 was analysed by radioimmunoassay of its methoximate derivative using an antiserum (supplied by Dr R.W.Kelly, MRC reproductive biology unit Edinburgh).[12] $LTC_4/D_4/E_4$ were measured by radioimmunoassay using the Amersham kit (Amersham International PLC, Amersham, Bucks, U.K.). This antibody to LTC4 cross-reacts with LTD_4 and LTE_4. LTB_4 was assayed by neutrophil chemokinesis after high performance liquid chromatography (HPLC)[13] and 12-HETE by gas chromatography/mass spectrometry (GC–MS).[14]

Statistical analysis of PGE_2 and $LTC_4/D_4/E_4$ levels was performed using two-way analysis of variance.[15] The paired *t*-test was applied to $LTC_4/D_4/E_4$ levels at time zero and 6 h.

RESULTS

Immediately after removal of weights the skin was indented by the rod but there was no oedema or erythema. Between 3 and 4 hours later the test sites became erythematous with increasing oedema. After 6 h erythematous tender palpable swellings > 2 cm in diameter developed at the pressure sites in all patients (Fig. 2). There was no evidence for increased cyclooxygenase activation; PGE_2 concentrations were 14·6 +/− 3·4, 16·9 +/− 3·4 and 17·6 +/− 4·2 ng/800 μl (mean +/− SEM, $n=6$, ng/800 μl) for the control, zero and 6 h sites respectively. Although measurements for $LTC_4/D_4/E_4$ were higher after pressure challenge (Table 1), the difference did not reach statistical significance. We found no consistent evidence for chemokinetic activity corresponding to LTB_4 in exudates purified by reverse phase HPLC in four patients. Levels of 12-HETE in exudate from two patients measured by GC–MS, showed no change 6 h after pressure challenge (mean 45 ng/800 μl) compared to the control unchallenged site (mean 58 ng/800 μl).

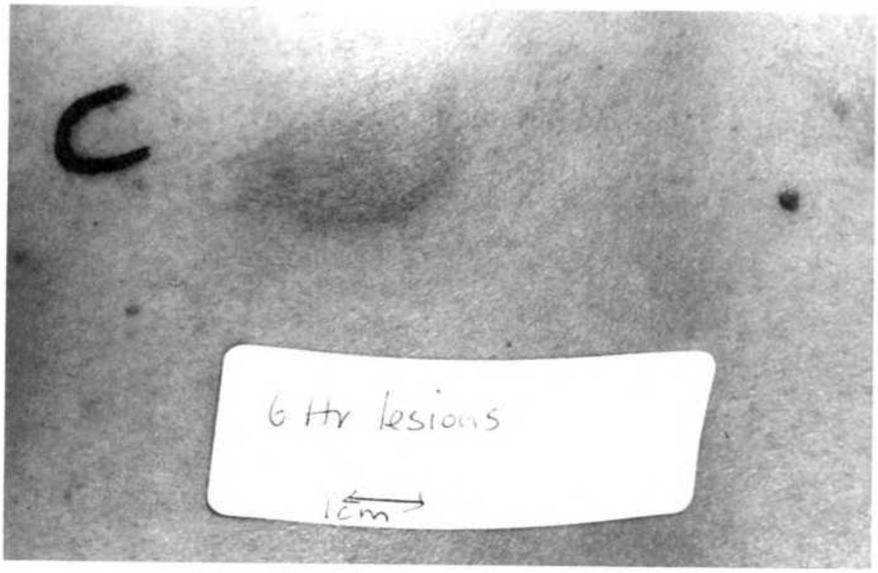

FIGURE 2. Experimentally induced pressure swelling at 6 h.

TABLE 1. Levels of leukotrienes $C_4/D_4/E_4$ found in skin chamber exudate in six patients with delayed pressure urticaria (pg/800 μl)*

Patient number	No pressure applied (control site)	Immediately after pressure removed (0 h)	6 h after pressure removed (6 h)
1	ND	80	210
2	106	208	212
4	< 72	224	272
5	< 72	252	212
7	211	176	292
8	256	80	212
Mean and standard error	143 ± 38 ($n = 5$)	188 ± 30 ($n = 6$)	240 ± 17 ($n = 6$)

* Level of detection 72 pg/800 μl.
ND = not determined.

DISCUSSION

We have been unable to demonstrate any change in prostaglandin E_2, the 5-lipoxygenase peptidyl-leukotrienes $LTC_4/D_4/E_4$, LTB_4 or 12-HETE during the development of lesions in DPU. It seems unlikely, therefore, that increased metabolism of arachidonic acid to eicosanoids plays a major part in the development of DPU lesions, either initially after pressure or at a later stage in association with infiltrating inflammatory cells. The lack of increased PGE_2 release is consistent with the failure of non-steroidal anti-inflammatory drugs to be of benefit in DPU.[1]

It has been reported that an increased chemoattractant activity showing the chromatographic properties of LTB_4 was present in lesional skin,[16] but we have been unable to confirm this. We were also unable to demonstrate a significant increase in 5-lipoxygenase transformation of arachidonic acid to the peptidyl-leukotrienes. Although this study of the 12-lipoxygenase pathway was limited to two patients, the evidence does not suggest activation of this pathway in DPU lesions.

The unavailability of suitable specific inhibitors or antagonists to lipoxygenase enzymes or their products makes it impossible to substantiate our conclusions in a clinical setting. Corticosteroids can be effective in the treatment of DPU[1] but it is uncertain whether they work through inhibition of phospholipase A_2, with a subsequent reduction in eicosanoid levels.

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Seasonal variation of skin resistance to irritants

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SUMMARY

In order to investigate a possible seasonal variation in the skin response to irritants, 17 healthy volunteers were patch tested with sodium lauryl sulphate (SLS) and nonanoic acid in the winter and summer. The response to these irritants was quantified by visual scoring, measurement of transepidermal water loss, measurement of electrical conductance indicating the hydration state of the superficial epidermis, measurement of blood flow by laser Doppler and measurement of oedema by ultrasound A-scan. Significantly stronger reactions to SLS were found during the winter than the summer as indicated by visual scoring and by measurements of transepidermal water loss, whereas no significant seasonal variation was found in the response to nonanoic acid. A decreased hydration state of the epidermis of unexposed skin was found during the winter, and we believe this to be responsible for the increased susceptibility to SLS during this season.

The possibility of a seasonal variation in the sensitivity of normal skin to irritants has been investigated by several workers. The influence of air moisture on chapping of normal skin has been reported[1] and seasonal variations in contact dermatitis have been demonstrated[2] with a maximum incidence in Denmark between October and March. Using the DMSO-test, a significantly increased response to DMSO has been demonstrated in the winter compared to the summer.[3] A marked seasonal variation has also been shown for irritant reactions to propylene glycol, the incidence being greatest in the winter months.[4,5]

This study was undertaken to investigate further the possible seasonal fluctuations in the sensitivity of normal non UV-exposed human skin to irritants, and the responses objectively measured by non-invasive methods.

METHODS

Seventeen healthy volunteers participated in the study (10 males, seven females; mean age 27 years, age range 25–38). Informed consent was obtained from all volunteers, and the study was approved by the local ethical committee.

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The flexor side of the upper arm was chosen as test region and closed patch tests applied using large Finn chambers (diameter 12 mm, Epitest®, Helsinki, Finland) on Scanpore tape. Water with 2·5% sodium lauryl sulphate (SLS, quality Ph Eur) and 20% nonanoic acid (NON, quality Merk) in propanol were used as irritants.[6] Propanol, sterile water and empty chambers served as controls. The volunteers were tested in the winter months (February–March) and retested in the summer (August–September). Due to technical problems only 15 subjects were retested with SLS, while all other tests were performed on all 17 subjects. Each subject had test chambers on both arms, two and three respectively on each arm. The placement of the chambers was randomized and blind to the investigator. Test chambers were removed after 24 h, and the reactions evaluated 30 min after removal.

Visual scoring was performed according to the following scale: 0, no reaction; 0·5, scaling or very weak erythema; 1, weak erythema, possibly slight infiltration; 2, marked erythema, infiltration, possibly vesicles and crusting; 3, marked erythema, infiltration, possibly vesicles, bullae, pustules and/or pronounced crusting.

Transepidermal water loss (TEWL) was measured by an evaporimeter (Servo Med® EP1).[7] The probe included an open chamber of 12 mm in diameter, and was mounted with sensors for determination of temperature and relative humidity. The TEWL is calculated automatically and expressed in g/m^2 per h. The reading was performed 30 s after application of the probe onto the skin, when the level is stabilized.[8] Probe protection cover was used.

Electrical conductance indicating the hydration state of the superficial epidermis was measured by a Skion-100® high frequency (3·5 MHz) hydrometer.[9] The probe is constructed with two concentrically arranged brass electrodes, applied to the skin surface with a standard pressure of 30 g. The recording time is 3 s and the conductance is registered and displayed digitally as reciprocal impedance (1/μohm).

A laser Doppler flowmeter (Periflux Pf$_2$®) was used for measuring the cutaneous blood flow.[10] The light from the laser is led by an optical fibre to the skin surface. The Doppler-shifted light is backscattered from moving blood cells, while unshifted laser light is backscattered from stationary tissues. The instrument was adjusted to a bandwidth of 12 kHz and again at 10. The output signal was expressed in relative and dimensionless blood flow values (a.u.). The laser Doppler probe was gently held onto the skin to avoid vascular compression, and the reading was performed as average values after stabilization.

Skin thickness was measured by a 20 MHz A-mode ultrasound scanner (Dermascan A®)[11] and expressed in mm. An acoustic velocity of 1580 m/s was used.[12]

All visual scorings and instrumental recordings were performed by the same investigator. All measurements were expressed as the mean value of three recordings. Measurements were performed with the arm level with the heart. During the winter period subjects rested approximately 15 min before measurements were performed, and in the summer the rest period had to be somewhat longer, to avoid problems with sweating. The laboratory temperature was kept in the range 19–21°C in the winter and 20–22°C in the summer, and the relative humidity was 35–45% in the winter and 50–75% in the summer period. Air convection or any disturbances in the laboratory were avoided as far as possible.

The Wilcoxon matched-pairs test was used when comparing winter and summer recordings. The chosen level of significance was $P < 0{\cdot}05$.

RESULTS

The results from the visual scoring of the patch test reactions and from the recordings of TEWL, hydration state of the epidermis, blood flow and oedema of the skin are demonstrated in

Tables 1 and 2. The number of subjects with a visual scoring of 0–3 is shown in Table 3. The visual scoring of the patch test reactions demonstrated significantly stronger reactions to SLS during the winter than during the summer period ($P<0{\cdot}05$). The NON patch test reactions tended towards the same pattern, but this finding was not significant.

TABLE 1. Irritant patch test reactions

		Winter	Summer	
Visual scoring (score)	SLS	2 (0·5–2)	1 (0·5–1)	$P<0{\cdot}05$
	NON	2 (0·5–2)	1 (1–2)	NS
TEWL (g/m^2 per h)	SLS	29·6 (14·9–55·0)	17·5 (14·8–26·2)	$P<0{\cdot}01$
	NON	9·2 (6·9–11·8)	6·8 (5·7–10·1)	NS
Electrical conductance (1/μohm)	SLS	60 (6–144)	45 (25–108)	NS
	NON	3 (1–4)	5 (5–7)	$P<0{\cdot}01$
Blood flow (a.u.)	SLS	58 (36–75)	32 (22–50)	NS
	NON	55 (37–80)	48 (39–72)	NS
Skin thickness (mm)	SLS	1·28 (1·15–1·52)	1·23 (1·15–1·39)	NS
	NON	1·26 (1·13–1·46)	1·23 (1·15–1·35)	NS

Irritant patch test reactions in the winter and summer periods. Median values and 25/75% percentiles of visual scoring, TEWL, electrical conductance, blood flow and skin thickness. The irritants used were sodium lauryl sulphate (SLS) and nonanoic acid (NON).

TABLE 2. Control patch test reactions

		Winter	Summer	
Visual scoring (score)	Pro	0 (0–0)	0 (0–0)	NS
	Water	0 (0–0)	0 (0–0)	NS
	Empty	0 (0–0)	0 (0–0)	NS
TEWL (g/m^2 per h)	Pro	6·7 (5·4–8·9)	5·7 (5·0–8·1)	NS
	Water	9·1 (7·9–11·2)	9·8 (8·3–11·6)	NS
	Empty	8·0 (6·5–9·0)	6·9 (6·2–9·2)	NS
Electrical conductance (1/μohm)	Pro	5 (3–8)	12 (11–24)	$P<0{\cdot}01$
	Water	17 (14–27)	24 (15–39)	$P<0{\cdot}05$
	Empty	24 (14–39)	44 (34–55)	$P<0{\cdot}01$
Blood flow (a.u.)	Pro	8 (8–11)	8 (8–12)	NS
	Water	10 (8–17)	16 (11–26)	NS
	Empty	9 (9–16)	9 (8–13)	NS
Skin thickness (mm)	Pro	0·96 (0·86–1·09)	1·00 (0·89–1·31)	NS
	Water	1·02 (0·92–1·22)	1·00 (0·92–1·12)	NS
	Empty	1·02 (0·85–1·13)	0·97 (0·89–1·06)	NS

Vehicles and control patch tests in the winter and summer periods. Median values and 25/75% percentiles of visual scoring, TEWL, electrical conductance, blood flow and skin thickness. The controls used were propanol (pro), sterile water (water) and empty chamber (empty).

TABLE 3.

Visual scoring	SLS ($n=15$) Winter	SLS ($n=15$) Summer	NON ($n=17$) Winter	NON ($n=17$) Summer
0	2	1	0	3
0·5	2	4	4	1
1	2	9	4	8
2	9	1	8	4
3	0	0	1	1

Number of subjects with visual reaction 0, 0·5, 1, 2, 3 in the winter and in the summer period.

The damage of the skin barrier function due to SLS, as measured by TEWL, was significantly more severe during the winter than in the summer ($P<0·01$) (Fig. 1). For NON patches the difference between winter and summer TEWL recordings was not significant. No significant differences were found between winter and summer values for any of the control patches.

SLS patch test areas showed a higher hydration state in the winter than in the summer period, but this finding was not significant. For NON patches and for all control patch test areas the hydration state of the superficial epidermis was significantly lower in the winter period when compared with recordings in the summer ($P<0·01$).

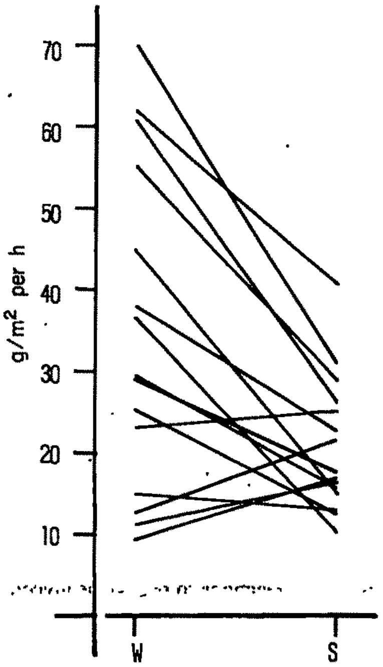

FIGURE 1. Transepidermal water loss (TEWL) in sodium lauryl sulphate (SLS) patches for individual subjects in winter (W) and summer (S) months. $n=15$.

The blood flow of the skin and the skin thickness showed no significant differences between winter and summer values, although the recordings of blood flow from the irritant patch test areas inclined toward lower values in the summer.

DISCUSSION

The two irritants used in the study have irritated the skin differently. SLS has been shown to damage the skin-barrier function, while NON has only a minor effect.[6] The fact that the seasonal variation was significant only for SLS patches implies that epidermal factors are responsible for the impaired skin resistance during the winter. Also with SLS the inflammatory response (blood flow and oedema) was without significant seasonal variation, while the difference in the skin-barrier function was found to fluctuate significantly with the season.

The variation in the skin sensitivity in the different seasons could be explained by the low outdoor temperature and the low relative humidity in the winter, which may lead to a decreased ability of the stratum corneum to retain water.[13] This hypothesis is supported by the findings in this study of a significantly decreased hydration state of the superficial epidermis, as measured by electrical conductance, during the winter. A low hydration state may be an important factor predisposing to impaired skin-barrier function and to toxic eczema.

Because of the increased environmental relative humidity during the summer period lower TEWL values might be expected during this season as compared to the winter period.[7] The present values for control patches with empty chambers were lower during the summer, but not significantly different from those in the winter. Also, during the summer the skin temperature may be higher than during the winter which results in increased TEWL values,[14] thus counteracting the influence of the changes in humidity. When comparing the different non-invasive methods for measuring irritant response and normal skin, it is obvious that parameters of hydration as measured by TEWL and electrical conductance show a more pronounced seasonal variation than the parameters of inflammation. No significant seasonal difference was found for blood flow, although laser Doppler recordings are also influenced by skin temperature. Ultrasound measured structural parameters and thickening due to oedema formation. Our results in this study demonstrated that the seasonal variation of these was not significant.

The seasonal difference in skin response is relevant when testing the skin for both irritant and allergic responses. As found in an earlier study,[2] false positive responses to allergens can be expected more frequently during the winter months due to the seasonal variation in the hydration and barrier function of the skin.

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Complement C3 proteins in psoriasis

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SUMMARY

A new polymorphism of the complement factor C3 in human plasma was demonstrated by isotachophoresis in agarose gels followed by immunodetection with rabbit anti-human $C3_c$ and $C3_d$ immunoglobulins. Four bands were detected in the immunoprint of freshly drawn EDTA-plasma, which were $C3_{s1}$, $C3_{s2}$, $C3_{f1}$ and $C3_{f2}$. At least four additional C3 components in Mg^{2+}-zymosan activated plasma were present, which were $C3_{b1}$ to $C3_{b4}$. The different forms of C3 in frozen and thawed heparin-plasma from 20 patients with psoriasis and 20 healthy individuals were studied from the immunoprint. The total content of C3 components was 29% greater in the patients with psoriasis than controls. The major difference was in the $C3_b$ components which were increased by 46%. In psoriatic patients, the two slow C3 components $C3_{s1}$ and $C3_{s2}$ were increased by 24 and 56% respectively, when compared with controls. The two fast C3 components $C3_{f1}$ and $C3_{f2}$ were decreased to 29 and 37%. The results suggest a direct involvement of the complement factor C3 in psoriasis.

Complement components are important factors in the defence against pathogenic micro-organisms. The third component of complement, C3, plays a pivotal role in this respect. Activation of the complement system leads to the formation of the opsonin $C3_b$ and the anaphylatoxin and inflammation-inducing fragment $C3_a$ and is the first step in the formation of the cytolytic membrane attack complex.[1] The activation of C3 *in vitro* can be induced by Mg^{2+}-zymosan. Physical changes such as freezing and thawing can also induce activation.[2]

The human C3 gene has been mapped to chromosome 19.[3] Beside several rare allelic variants, two major forms of C3, separated electrophoretically, have been described and are $C3_s$ (slow) and $C3_f$ (fast) according to their relative electrophoretic mobility.[4,5] Recently a different polymorphism with a close relation to $C3_f$ based on the reactivity of a mouse monoclonal antibody HAV 4–1, has been described.[6] Genetic polymorphism of human C3 has also been demonstrated by analysis of restriction fragment length polymorphism at the DNA level.[7]

The electrophoretic technique currently used for the analysis of C3 has a low resolving capacity. Fractionation of C3 components by isoelectric focusing (IEF) produces a diffuse zone. Isotachophoresis (ITP) in agarose gels, a technique developed in this laboratory, has the resolving capacity of IEF with the advantage of pH and ionic strength flexibility, permitting the separation of genetic variants of several plasma proteins.[8] This paper presents the results of the comparison of the analysis of C3 components in freshly drawn EDTA-plasma and in Mg^{2+}-zymosan activated plasma.

METHODS

Human plasma samples from healthy donors were supplied by the Blood Transfusion Service at the Karolinska hospital and from patients with psoriasis during active flare of their disease by the Department of Dermatology. Plasma was taken from 20 patients, 17 of whom had psoriasis vulgaris, and two of whom had psoriatic arthropathy. Of the remaining three patients one had erythroderma, one had guttate psoriasis and one pustular psoriasis. The patients were treated with topical remedies—mostly dithranol. Most had had psoriasis for more than 2 years. The average age was 57 ± 14 years. The blood samples were centrifuged at 0°C, and the plasma was divided into 1 ml portions and stored at −20°C.

Ampholytes and paper electrode strips were from LKB (Bromma, Sweden), agarose, electrode strips and paper sample applicators from Pharmacia (Uppsala, Sweden), rabbit immunoglobulins (Igs) against human C3 antigens from Dakopatts (Copenhagen, Denmark), gelbond from FMC Bioproducts (U.S.A.) and zymosan from Sigma (U.S.A). The flat bed electrophoresis apparatus used was FBE 3000 from Pharmacia (Uppsala, Sweden) and the gel scanner was ultroscanner XL2222 laser densitometer from LKB (Bromma, Sweden).

Activation of C3

Freshly drawn EDTA-plasma samples from healthy individuals were incubated for 0 or 15 min at 37°C in 10 mM $MgCl_2$ with 5% zymosan, pre-boiled in 150 mM NaCl, 5 mM phosphate buffer, pH 7·4.

Isotachophoretic analysis

The procedure was as previously described.[8] The plasma proteins were run towards the anode in 0·07 × 12 × 24 cm 1% agarose IEF, 12% sorbitol gels on a flat bed electrophoresis apparatus. The leading ion was 40 mM glutamic acid and the counter ion was 40 mM histidine. A mixture of ampholytes, 50% ampholines 4–6 and 50% 5–7, was used as spacers. The terminating ion was β-alanine. The leading ion, the counter ion and 1·4 ml of the spacer mixture, were added to the agarose suspension (20 ml) at 75°C and the gel was cast on 24·5 × 12·6 cm gelbond film. The cathodic solution was 1 M β-alanine and 0·25 M histidine containing bromophenol blue. The anodic solution was 0·25 M histidine and 40 mM glutamic acid. Ten millilitres of each of these solutions were embedded in 1 × 0·6 × 23 cm electrode strips and placed on the long edges of the gel. The electrophoresis was run for about 1 h at 5 W until the isotachophoretic front reached 1·5–2 cm from the cathode. Then, the 8 μl samples, one part fresh or thawed plasma to five parts histidine 80 mM, were applied on the paper sample applicators, cut to half its width (1 × 0·25 cm), placed behind the front and perpendicular to it. The run was carried out at 10 W (300–900 V) until the front reached the anode after about 3–4 h at 10°C. The protein bands were visualized by immunotechniques (see below) or with Coomassie Brilliant Blue (CBB).

Immunoblotting

The proteins were transferred to a nitrocellulose (NC) filter by overlying the gel for 20 min at room temperature with a stack consisting of a wet NC filter, a wet blotting paper, eight layers of dry blotting paper (Munktell, Sweden), a glass plate and on the top a 5 kg weight. The immunological detection of proteins on NC filters was done essentially according to Towbin *et al.* as described in Andrews,[9] except that 5% dry defatted milk in 0·9% NaCl 10 mM Tris-HCl buffer pH 7·4 (TBS) was used instead of bovine serum albumin. The Igs were diluted 1 : 1000 in 5% dry defatted milk, 10% swine serum in TBS. The peroxidase staining was done according to Hawkes *et al.* as described in Andrews.[9]

Immunoprint

The identification of the C3 components after the separation by ITP was done by overlying a new gel, 0·1 cm thick 1% agarose C gel in 75 mM Tris-barbital buffer, pH 8·6, containing the antibodies rabbit anti-human $C3_c$ immunoglobulins (with a titre of 1200 μl per μg antigen), 2 μl/cm², for 20 min at 20°C. The immunoreplica was incubated 2 h at 20°C or overnight at 4°C in 1 l 0·9% NaCl, washed twice in 0·9% NaCl and stained with CBB. The ITP gel was washed three times, 5 min each, with NaCl 0·9%, wiped on the surface with a wet soft paper and then fixed and stained with CBB.

Quantification

The protein content in the stained bands was calculated from the densitometric measurements obtained with a gel scanner.

RESULTS

The result of the isotachophoretic analysis of plasma C3 components is shown in Figure 1. Four bands of C3 stained after immunoblot with anti-human $C3_c$ in non incubated samples, whereas at least four additional components appeared after 15 min of incubation with Mg^{+2}-zymosan at

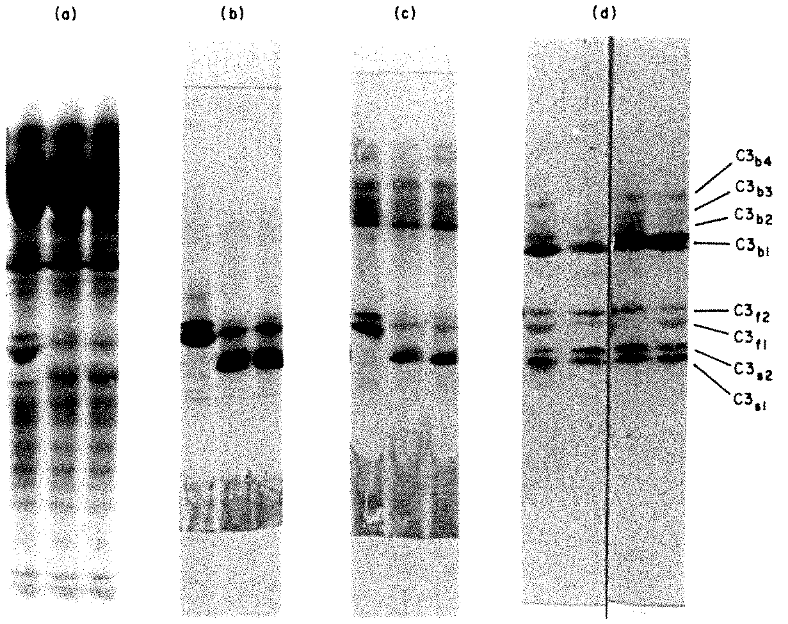

FIGURE 1. Isotachophoresis of EDTA-plasma samples from healthy individuals. (a) CBB stain. (b) Immunoblot stained with anti-human $C3_c$. (c) Immunoblot as in (b). The plasma samples were incubated with Mg^{2+}-zymosan for 15 min at 37°C before the analysis. (d) Immunoprint obtained with anti-human $C3_c$ after isotachophoresis of frozen and thawed heparin-plasma samples.

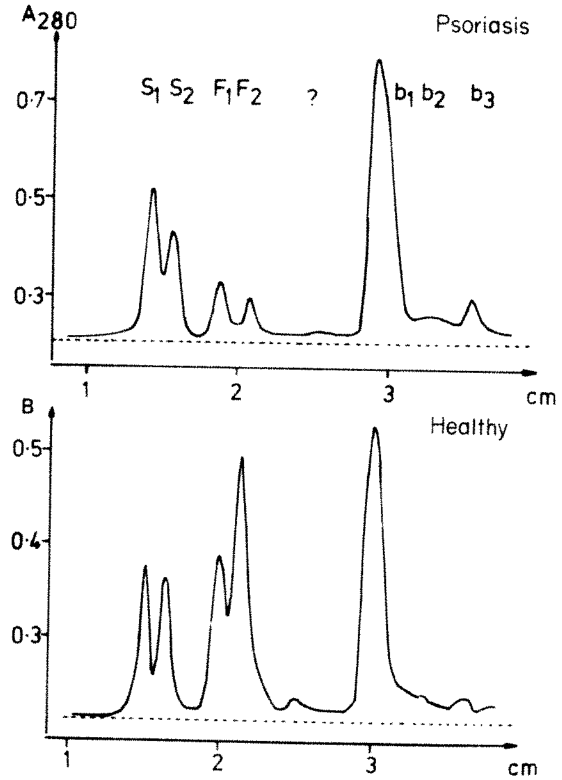

FIGURE 2. Isotachophoresis of frozen and thawed plasma proteins. Scanning of the immunoprint (by apposition of an antibody containing gel, 2 μl/cm^2 rabbit anti-human C3$_c$ with a titre of 1200, o of an ITP gel similar to that shown in Figure 1. A. Psoriasis patient. B. Healthy individual.

37°C or after freezing and thawing. Similar results were obtained after s immunoblots with anti-human C3$_d$ (not shown).

The results of the scanning of the immunoprint with anti-human C3$_c$ obtain isotachophoretic analysis of two frozen and thawed plasma samples, from a health from a patient with psoriasis, are shown in Figure 2. The results of the integrated d analysis of an immunoprint with anti-human C3$_c$ after isotachophoretic analysis (and thawed plasma samples are shown in Figure 3 indicating variation in the co components between psoriatic and healthy subjects.

There are at least four different C3 components in human plasma. Upon activatio Mg^{2+}-zymosan at least four C3$_b$ components are formed. In the frozen and tha samples analysed the total content of C3 components was increased in plasma fron The major difference was found in the C3$_b$ components. The ratio of C3$_b$ between p control was 1·34 ± 0·06 (SEM). The proportions of the two slow C3 components, d C3$_{s1}$ and C3$_{s2}$, was higher in psoriatics than in controls and the corresponding 1·24 ± 0·07 and 1·56 ± 0·07, respectively. The proportion of the two fast C3 comp and C3$_{f2}$ was higher in plasma samples from healthy donors. The ratios (psoriasis were 0·29 ± 0·01 and 0·37 ± 0·02, respectively.

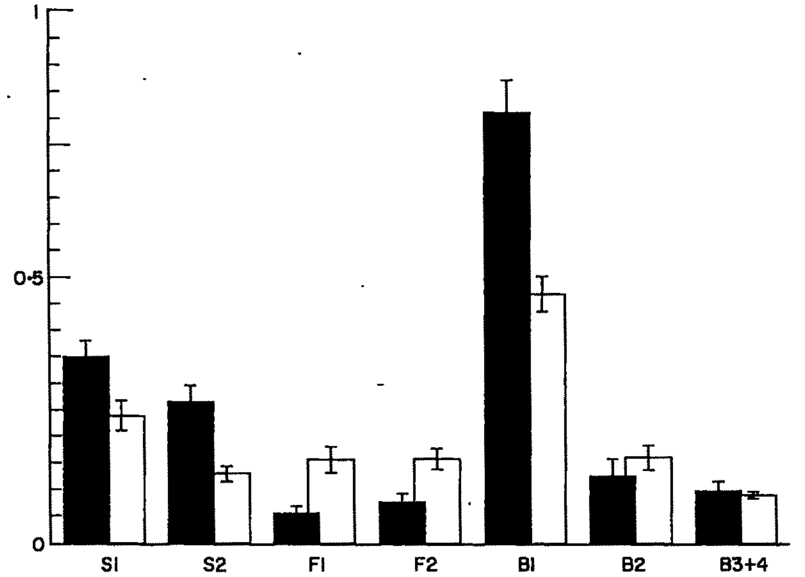

FIGURE 3. Integrated densitometric analysis of an immunoprint with anti-human $C3_c$ after isotachophoretic analysis of 40 frozen and thawed plasma samples: 20 from psoriasis patients and 20 from healthy individuals. □, control; ■, psoriasis; |—|, SEM. The ordinate represents the integrated area (A_{280} × length in mm).

DISCUSSION

The technique illustrated here permits a rapid and direct separation of at least four different major C3 forms which we have called $C3_{s1}$, $C3_{s2}$, $C3_{f1}$ and $C3_{f2}$, since they appear as duplets of the slow and fast allotypes referred to in the literature. The four major $C3_b$ components, which we have called $C3_{b1}$ to $C3_{b4}$, might represent the activation products of the corresponding C3 components.

The larger content of $C3_b$ in the samples from psoriatics, concomitant with the smaller content of $C3_f$ components, may indicate a faster degradation of the latter in psoriasis when compared with controls. However, it has been reported that different allotypes of mouse C3 degrade at different rates where the slow electrophoretic form degrades faster.[10] It is of interest that $C3_b$ has been shown to stimulate the proliferation of B-lymphocytes.[11] Results obtained in our laboratory also show that $C3_b$ has a keratinocyte stimulating effect.

A preponderance of $C3_f$ has been demonstrated in patients with hypercholesterolemia,[12] rheumatoid arthritis,[13] ischaemic heart disease with essential hypertension[14] and in old people with arteriosclerosis.[15] A hypomorphic variant of $C3_f$ has also been described associated with the immune complex type of glomerulonephritis and arthritis.[16] It has also been reported that $C3_f$ has a higher capacity than $C3_s$ for binding to receptors on mononuclear cells.[17] An inflammatory reaction occurs in psoriasis, and complement factor proteins are involved. Deposits of complement factor C3 in the parakeratosis seen in psoriasis have been regarded as a secondary phenomenon to the hyperproliferation.[18] Recently, several groups have described C5-desArg in psoriatic scales and inferred that its deposition is responsible for the neutrophil chemotaxis.[19]

The development of suitable analytic techniques has facilitated the study of the role of the different genetic variants of C3 in psoriasis and the involvement of C3 in cell proliferation.[20] At present, no data are available on C3 receptors on epidermal cells in psoriasis, although there are at least three such receptor proteins reported in various cells.[21–23] An anti-$C3_d$ antibody that binds to the epidermal basement membrane has been reported.[24] Preliminary experiments using antibodies to the CR-1 and CR-2 receptors which bind $C3_b$ and $C3_d$, respectively, indicate the expression of these receptors in the human epidermis. The availability of monoclonals against various epitopes of the complement proteins and their receptor proteins will increase our knowledge concerning the presence and function of complement factors with regard to skin diseases and in particular psoriasis.

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The bacteriology of chronic venous ulcers treated with occlusive hydrocolloid dressings

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SUMMARY

The bacterial flora of chronic venous ulcers treated with an occlusive hydrocolloid dressing were studied over a period of 8 weeks. A novel exudate sampling method was used in an attempt to isolate anaerobic bacteria. The flora was generally stable. Once a species was present, it remained with the exception of *Pseudomonas*, which appeared to be inhibited by the dressing. Twelve out of 20 ulcers contained anaerobic bacteria and healing did not appear to be impaired by the presence of any particular species of bacteria.

Chronic venous ulceration is a condition affecting many thousands of sufferers throughout the U.K.[1] A large proportion of these patients have their ulcers dressed by community nurses or at general practitioners' surgeries.[2]

It has been claimed that 'occlusive' dressings promote rapid wound healing by preventing dehydration and scab formation, thus allowing advancing epithelium an uninterrupted passage across newly formed granulation tissue.[3] However, there is concern that a moist environment may lead to bacterial proliferation and wound infection[4] and several adverse reports appear to confirm this view[5,6].

Recently, a new dressing, described as an 'occlusive hydrocolloid' ('Granuflex' Squibb Surgicare, also known as 'Duo Derm' in countries other than the U.K.) has become available for the treatment of granulating wounds. Although clinical studies[7,8] have not reported infection as a problem, the fact that the dressing is impermeable to oxygen[9] and to bacteria,[10] and that it produces an offensive-smelling exudate when in use has led us to question its effect on the bacterial flora and especially the effect on anaerobes.[11] The purpose of this study was to investigate changes in the bacterial flora that occurred in chronic venous ulcers treated with this hydrocolloid dressing.

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METHODS

Patients

Twenty venous ulcers were studied over an 8-week period. The basic information concerning our patients is shown in Table 1. The patients were all assessed at a leg ulcer clinic, and attended a weekly out-patient clinic where all the dressings, specimen collection and measurements were performed by one of us. All had chronic venous ulceration that was resistant to at least two previous conventional treatments and had no antibiotic treatment in the 2 weeks prior to the study.

Full blood counts, including haematology screen, urea and electrolytes, thyroid function tests, blood sugar and liver function tests were performed and no significant abnormalities were found. Significant arterial involvement was excluded by Doppler ultrasound studies. The study was approved by the hospital ethical committee and written informed consent was obtained from all subjects prior to the study.

TABLE 1. Sample characteristics

No. of ulcers studied	20
No. of patients	18
Sex: male	4
female	14
Average age (years)	70·7 (50–83)
Average ulcer size (mm^2)	994 (53–2961)
Average time present (months)	6·6 (1–13)

Dressing procedure

Prior to treatment, the ulcer was photographed and its outline traced. The ulcer was cleaned with sterile normal saline and the dressing applied according to the manufacturer's instructions. Where appropriate, granules were also used. The dressing was covered with a sterile dressing pad and compression was applied using either 'Venosan 2000' elastic compression stockings, (Credenhill Ltd, Ilkeston, Derbyshire, U.K.) or double layer shaped Tubigrip (Seton Products, Oldham, Lancs, U.K.). The patients were seen by a District Nurse 4 days later and the dressing changed if necessary. A written instruction sheet ensured that a standard procedure was used.

Microbiological procedures

Wound swab. Prior to the initial dressing and at each change, swabs were taken for culture of aerobic and anaerobic bacteria and placed in Stuart's medium.

Exudate sample. Each week, as the dressing was being removed, a measured amount of exudate (up to 1·0 ml) was collected in a sterile plastic pipette, and immediately placed into 8·0 ml of a specially prepared anaerobic transport broth.

Preparation of transport broth. The broth was made by adding 5 ng/ml of haemin (BDH) and 0·03% sodium formaldehyde sulphoxylate (BDH) (a reducing agent) to standard nutrient broth (Oxoid). Aliquots (8·0 ml) were place into sterile glass bijoux bottles, which were placed in a boiling water bath for 20 min with the caps loosened to blow off any oxygen. The caps were then closed tightly.

Culture of specimens. All swabs and exudate samples were plated out within 4 h of collection. None of the specimens were refrigerated. The swabs were plated on to whole blood agar containing naladixic acid, and on to MacConkey agar and incubated overnight at 37°C. In addition, swabs plated on to cetrimide agar were cultured overnight at 42°C to detect *Pseudomonas* species. Swabs plated on to whole blood and neomycin blood agar were cultured anaerobically at 37°C for at least 5 days. Broth containing exudate was plated on to whole blood agar, neomycin blood agar and NAT and NAV media[12] and incubated at 37°C for at least 5 days.

Viable counts. Viable counts of aerobic organisms were semi-quantitative only. Growth was measured as scanty (+), moderate (+ +), or heavy (+ + +), and the plates were read by the same observer each week.

Viable counts of anaerobic organisms were made from a known volume of exudate inoculated into a known volume of the specially prepared anaerobic broth. Serial dilutions of this mixture were then made into fresh anaerobic broths and a standard 0·02 ml loopful from each dilution was plated out. An average viable count was calculated from the dilutions where separate colonies could be counted.

Identification of organisms. Organisms cultured aerobically were identified using standard laboratory methods. Anaerobic organisms were identified by colonial morphology, Gram strain, strict anaerobic growth, sensitivity to metronidazole, sensititivity to novobiocin (Gram positive isolates); carbohydrate fermentation and gas liquid chromatography.

Analysis of results

Ulcer area (mm^2) was calculated from the acetate tracings using computerized planimetry. Healing rates were calculated from the slope of the log (area) against time plot as previously described.[11] Statistical analysis was by the Student's *t*-test.

RESULTS

Healing

In the 8-week period 5 out of 20 ulcers healed completely, while the average overall decrease in area was 58·3%. In the 15 ulcers which did not heal the average decrease in area was 44·4%. Eleven out of 20 had healed completely at 12 weeks.

Bacterial flora

The changes in aerobic bacterial flora are shown in Table 2. Of the species which were acquired, the striking feature was the appearance of *Streptococcus faecalis* in 10 out of 20 ulcers. In eight cases this was in the week following commencement of treatment.

The only species which was consistently lost was *Pseudomonas*. In each ulcer where it was present, there was a consistent decline (as measured semi-quantitatively) and with one exception, had disappeared within 4 weeks. The disappearance of the *Streptococcus* Group A from ulcer 8 followed treatment with oral erythromycin as it was felt that the risk to the patient of a potentially serious infection was too great. Anaerobic bacteria were cultured from 12 out of 20 ulcers studied (Table 3). Anaerobes were present at some time in 6/9 ulcers that healed completely.

TABLE 2. Changes in bacaterial flora (excluding anaerobes)

Ulcer No.	Pre-treatment flora	Species acquired (week)	Species lost (week)	Initial size (mm^2)	8-week size (mm^2)
1.	*Ps. stutzeri* *Staph. aureus* *Strep. faecalis*		*Ps. stutzeri* (1)	768	285
2.	*Ps. aeruginosa* *Strep.* Group G	Coag. neg. Staph. (2)	*Ps. aeruginosa* (2)	805	432
3.	*Ent. cloacae* *Kleb. oxytoca* *Prot. mirabilis* *Strep. faecalis*	*Staph. aureus* (2)	*Ent. cloacae* (2)	1538	1520
4.	*Staph. aureus*			293	0
5.	*Ps. aeruginosa* Coag. neg. Staph.	*Strep. faecalis* (1) *Prot. mirabilis* (1)	Coag. neg. Staph. (1) *Ps. aeruginosa* (3)	173	0
6.	*Ps. aeruginosa*	*Prot. mirabilis* (1) *Strep. faecalis* (1)	*Ps. aeruginosa* (5)	3071	0
7.	*Kleb. oxytoca*	*Prot. morganii* (4) *Staph. aureus* (8)		1267	0
8.	*Staph. aureus* *Strep.* Group A	*Strep. faecalis* (1)	*Strep.* Group A. (4)	1059	0
9.	*Prot. mirabilis*	*Strep. faecalis* (1)		1368	760
10.	*Prot. mirabilis*	*Strep. faecalis* (3)		53	14
11.	*Staph. aureus*	*Strep. faecalis* (1)		147	84
12.	*Ent. cloacae* *Staph. aureus*	*Strep. faecalis* (1)		286	174
13.	*Prot. mirabilis*	*E. coli* (2)		196	
14.	*Staph. aureus*	Coag. neg. Staph. (6)		1297	374
15.	*Staph. aureus* *Strep.* Group B			124	78
16.	*E. coli* *Ps. stutzeri*	*Kleb. oxytoca* (1) *Strep. faecalis* (1) Coag. neg. Staph. (6)	*Ps. stutzeri* (1)	750	373
17.	*Prot. mirabilis* *Ps. aeruginosa*	*Strep. faecalis* (1) *Kleb. aerogenes* (2) *Strep.* Group G (3)	*Ps. aeruginosa* (4)	2128	1258
18.	*Ps. aeruginosa*	Coag. neg. Staph. (2) *Kleb. aerogenes* (2) *Strep. faecalis* (4)	Coag. neg. Staph. (5)	3196	1668
19.	No growth	*Staph. aureus* (2) *Strep.* Group B (3)		1065	923
20.	*Staph. aureus* *Strep. faecalis*	*Cit. freundii* (8)	*Staph. aureus* (7)	669	697

DISCUSSION

The principle of 'moist wound healing,' first described in an experimental wound,[13] and later confirmed in man,[14] appears to offer advantages in terms of the rate at which epithelialization occurs in a granulating wound. However, the trapping of moisture by an occlusive dressing on the intact skin can produce an explosive proliferation of bacteria.[15,16] The introduction of

TABLE 3. Anaerobic bacteria

Ulcer no.	Species (week)
3	*Peptococcus magnus* (6)
5	*Peptococcus asacchrolyticus* (1)
7	*Peptococcus asacchrolyticus* (1)
	Peptococcus magnus (1)
9	*Peptococcus magnus* (2)
11	*Peptococcus magnus* (6)
13	*Peptococcus magnus* (2)
	Peptococcus prevotii (3)
14	*Peptococcus magnus* (2)
	Peptococcus niger (2)
	Bacteroides melaninogenicus (2)
	Peptococcus asacchrolyticus (3)
16	*Peptococcus magnus* (1)
17	*Peptococcus prevotii* (1)
	Peptococcus magnus (8)
18	*Peptococcus niger* (3)
	Peptococcus prevotii (3)
	Peptococcus magnus (4)
19	*Bacteroides distasonis* (1)
	Bacteroides fragilis (6)
	Peptostreptococcus anaerobius (6)
20	*Peptococcus niger* (6)

polythene dressings semi-permeable to water vapour[17] did allow clinical application of this principle and the demonstration that the fluid under the dressing contained active neutrophils[18] reduced anxieties about infection although doubts were still expressed.[19] Occlusive hydrocolloids are impermeable to water[20] and the dressing particles absorb wound exudate forming a gel that fills the wound cavity. It is claimed that this colloid gel produces a sorption gradient for soluble components within the exudate, thus allowing the removal of toxic components produced by cellular and bacterial destruction.[20]

The main finding of this study, that ulcers treated with hydrocolloid dressing appear to maintain a fairly stable microbial flora, has been reported previously[21] although one study[22] found that fewer types of organisms were present at each dressing change. Similar results have been described where other dressings have been used. However, comparison of studies is difficult because of widely differing methodology and measurement of the bacterial flora.

Daltrey and Cunliffe[23] compared the use of 20% benzoyl peroxide with eusol and paraffin, although no details of the dressing technique are given. They found that most ulcers were colonized by several different organisms, and although they were able to eradicate *Staphylococcus aureus* with the application of the peroxide, they concluded that neither preparation showed any strong antibacterial effect, a finding confirmed by others.[24] No details of healing were provided.

In a study comparing occlusive dressings with either non-adherent dressing (NA) or Flamazine,[25] it was found that 80% of the venous ulcers sampled grew more than one organism. In the Flamazine and NA groups bacterial contamination continued throughout the study; the authors report that the presence of organisms did not appear to delay healing, and they questioned any attempt to achieve a bacteria-free ulcer.

Thirty-one venous ulcers were randomly assigned to treatment with either dextranomer ('Debrisan') or streptokinase–streptodornase ('Varidase').[26] Reduction in infection as evaluated by clinical inspection of the wounds, was reported in both groups, but the bacteriology remained much the same in about half of the patients studied. In a further study evaluating the use of Debrisan on moist, infected and profusely draining ulcers which were treated for an average of 4 weeks,[27] it was found that, although the types of organisms decreased (from a mean of 3·3/ulcer to a mean of 1·5), the numbers present (as measured on a log scale) were the same, or increased, in 13/30 ulcers.

To examine the effect of systemic antibiotics, Alinovi *et al.*[28] randomly assigned patients to receive either a standard ichthammol bandage plus elastic bandage, or the standard treatment plus a 10 day course of an appropriate oral antibiotic, based on culture results. After 20 days 12 out of 55 ulcers had healed; of the remaining 43, 30 had either no change in their flora ($n = 13$) or had acquired new organisms ($n = 17$). There was no significant difference between the groups in relation to either healing rates, or loss of bacterial species. They conclude that the changes in bacterial flora are secondary as the ulcer heals rather than the healing being the result of the change in flora. Similar findings are also reported by Alper[29] who found that ulcers treated with polyurethane film ('OpSite') healed in the face of 'gross bacterial contamination'.

In contrast, a statistically significant reduction in *S. aureus* and *Pseudomonas aeruginosa* has been reported[30] in ulcers treated with cadexomer iodine ('Iodosorb') when compared to a control group being treated with either hydrogen peroxide or potassium permanganate, but these results must be treated cautiously as the bacteria were only quantified on a +, + +, + + +, scale, and the numbers involved are very small.

The apparent anti-Pseudomonal effect of the dressing has been reported previously.[31,32] Three possible mechanisms have been suggested: (i) the sensitivity of *Pseudomonas* to dessication, (ii) the effect of the pH under the dressing, (iii) the exclusion of oxygen by the dressing. In this study *Pseudomonas* disappeared within 4 weeks in 6 out of 7 ulcers. The remaining subject revealed that he had been lifting the dressing each morning to squeeze out excess exudate to prevent leakage during the day, thus allowing oxygen in. When this practice was discontinued the *Pseudomonas* was absent 7 days later. This may explain the apparent discrepancy between our findings and those of Handfield-Jones *et al.*[33] who did not observe this effect. Full suppression of the *Pseudomonas* did not occur until week 4, and this coincided with the point at which the dressing could be left undisturbed for 7 days. The appearance of the *Streptococcus faecalis* was unexpected. Control swabs of the intact skin around the ulcer did not reveal the organism before treatment, though the organism may have been carried on the hands of the nurses. It is also possible that breakdown products of the dressing present in the exudate may, in some way promote, the growth of the *Streptococcus*.

The incidence of anaerobic bacteria was much greater than previously reported[21] and we feel that this is due to the method of specimen collection, as anaerobes are notoriously difficult to isolate without special care.[34] Their presence could not be correlated with poor wound healing; seven of the 11 ulcers which healed completely contained anaerobes at some time and we could not demonstrate any significant difference in the healing rates of ulcers that contained anaerobes as compared with those which did not ($P = 0·26$). The presence of a foul-smelling odour has been used as an indicator of the presence of anaerobic bacteria.[35] This is not possible with this dressing as the exudate always has an offensive smell when the dressing is removed, whether anaerobes are present or not. The cause of this odour is likely to be due to the breakdown products of gelatin contained within the dressing.

The effect of bacteria on the healing of a chronic wound is uncertain as it is difficult to identify which bacteria are pathogenic and which are not.[36] Nevertheless, there is increasing evidence that the presence of large numbers of many species of bacteria does not adversely affect healing[29,37–39], and the presence of bacteria does not seem to influence diagnosis, treatment or prognosis.[40] There seems to be no need to routinely send swabs of chronic leg ulcers for bacteriological culture. This would result in a considerable monetary saving, and help to prevent the inappropriate use of topical or systemic antibiotics[41] and the use of toxic chemicals that may delay wound healing.[42]

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Continuous wave dye laser therapy of port wine stains

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SUMMARY

A continuous wave dye laser emitting light at 577 nm has been used in the treatment of 100 patients with port wine stains. Subjective assessments in 92 patients revealed an excellent or good result in 63% and a fair result in 17%. There was a 5% incidence of hypertrophic scarring and a 5% incidence of post-inflammatory hyperpigmentation. Overall, 12% of patients experienced a poor result. The best results were obtained in older patients with purple port wine stains.

Port wine stains are benign vascular naevi consisting of mature ectatic dermal blood-vessels most of which are full of erythrocytes. There is an increase with advancing age in both the number of abnormal vessels and their ectasia. This is associated with a colour change of the naevi from pink to purple.[1] Lasers have been used in the treatment of port wine stains for over 20 years, initially with the ruby laser,[2] and subsequently the argon[3,4] and carbon dioxide lasers.[5,6]

The argon laser emits blue-green laser light, predominantly at wavelengths of 488 and 514 nm which, although selectively absorbed by oxyhaemoglobin, does not coincide with the absorption peaks of this molecule at 415, 542 and 577 nm. Moreover, there is competitive absorption of argon laser light by melanin at 488 and 514 nm. The carbon dioxide (CO_2) laser emits in the far infra-red part of the electromagnetic spectrum, at a wavelength of 10 600 nm and is selectively absorbed by tissue water, producing a non-specific thermal destruction of treated tissue. Both the argon laser and the CO_2 laser can produce satisfactory improvements in the appearance of port wine stains in 50–80% of cases with an incidence of scarring of up to 15%.[7]

The tunable dye laser has been developed more recently. This uses a suitable rhodamine dye and emits laser light at 577 nm which coincides with one of the absorption peaks of oxyhaemoglobin. There is less absorption by melanin. In theory, this laser should be a suitable and more specific treatment than either the argon or the CO_2 laser for port wine stains. A flashlamp-pumped pulsed dye laser with pulse durations varying between 20 and 360 μs has produced satisfactory results in the treatment of port wine stains.[8,9]

In a preliminary study using a continuous wave dye laser emitting at 577 nm and with ms or longer pulses, Cotterill[10] demonstrated that this laser was effective in the treatment of a wide range of vascular lesions, including port wine stains. We report our experience in the treatment of 100 patients with port wine stains using a continuous wave dye laser emitting at 577 nm.

METHODS

Over the past 4 years, 100 patients have received treatment with a continous wave Coherent CR599 argon pumped dye laser using a rhodamine dye to emit laser light at 577 nm. The details of the patients treated are shown in Table 1.

After obtaining informed consent, a trial area of the port wine stain was treated after local anaesthetic infiltration with 2% lignocaine. Eye protection was worn by both patients and operator. A 1 cm^2 test area was treated using the minimal blanching technique. The spot diameter on the skin was 2 mm with a power range of 0·3–0·6 W in most patients, and pulse durations of 0·2 to 2·0 s (energy fluence 10–32 J cm^{-2}). Contiguous or slightly overlapping spots were produced. The area treated was not dressed and a slight serous discharge developed a few hours after treatment, which was replaced by a crust. This separated after 2 weeks to leave a pink area which began to pale, in most patients, after 3 to 4 months. However, this process often took 2 years to reach maximum pallor following treatment.

The patients were reviewed 4–6 months after treatment to assess the response to therapy. Assessments were based on paling of the treated area, the development of scarring and pigmentary change. If any detectable paling occurred without scarring, treatment was continued. If scarring occurred, no further treatment was offered. Pigmentary changes were assessed with the patient in terms of the overall change in colour. If no changes occurred a further test area was performed at higher power or the patient was reviewed 6 months later for signs of paling of the test patch.

The results of laser treatment on port wine stains (PWS) were assessed on a 4-point scale: excellent—normal or near normal skin colour without scarring or pigmentary change; good—significant lightening of PWS ± hypopigmentation; fair—observable pallor of PWS of some cosmetic significance ± hypopigmentation, atrophic scarring; and poor—no change in colour of PWS, hypertrophic scarring and hyperpigmentation.

TABLE 1. Details of patients treated

No. = 100	
Female 70, Male 30	
Mean age 28·1 years (range 11–61)	
Colour of naevus	
Purple	51
Red	18
Pink	31
Site of naevus	
Face	86
Neck	2
Arm	7
Trunk	1
Leg	4
Previous therapy: 22 (Grenz rays, Thorium X, Tattoos)	

In practice, hypopigmentation or atrophic scarring was rarely seen without significant blanching. In addition, many patients found hypopigmentation ± atrophic scarring a considerable cosmetic improvement when compared to the untreated port wine stain.

Statistical comparisons between responding groups were made using the χ^2 test on non-parameteric data and the Student t-test on parametric data.

RESULTS

The results of dye laser treatment are shown in Table 2. Assessments were based on the overall appearance of all areas treated. All patients had at least a test area assessed and 16 patients had completed therapy. Seventy-six had a subtotal treatment of the port wine stain and eight were lost to follow-up. A good or excellent response occurred in 63% of patients, a fair response in 17% and a poor response in 12%. There was a 5% incidence of hypertrophic scarring and in 5% there was post-inflammatory hyperpigmentation.

Of the 16 patients who completed therapy, 12 had an excellent result, one a good result, two a fair result and one had a poor result. The final assessments in these patients had not changed from the initial assessments of the test area, except in one patient who had near normal skin colour after all treatments but developed a hypertophic scar on the upper lip after his last treatment. He was assessed as a poor result. The results of patients who had a satisfactory outcome after laser therapy to their port wine stain are shown in Figures 1–3. The notes of the eight patients lost to follow-up were reviewed and the assessments are shown in Table 3. These patients did not all show unduly poor results.

TABLE 2. Response to therapy

No. = 92 (8 lost to follow-up)	
Excellent	19
Good	44
Fair	17
Poor	12
Scarring	
Atrophic	14
Hypertrophic	5
Keloid	0
Pigmentary change	
Hypopigmentation	14
Hyperpigmentation	5

TABLE 3. Results in patients lost to follow-up

No. = 8		
Excellent	2	
Good	3	(1 'doesn't like needles')
Poor	3	(2 no effect, 1 hypertrophic scar)

TABLE 4. Excellent responders

No. = 19	
Female 14, Male 5	
Mean age 35·7 years	
Colour	
Purple	14
Red	4
Pink	1
Site	
Face	19
Previous therapy	5

TABLE 5. Good responders

No. = 44	
Female 35, Male 9	
Mean age 29·6 years	
Colour	
Purple	30
Red	7
Pink	7
Site	
Face	40
Neck	2
Arm	2

TABLE 6. Fair responders

No. = 17	
Female 9, Male 8	
Mean age 23·0 years	
Colour	
Purple	5
Red	4
Pink	8
Site	
Face	14
Arm	1
Trunk	1
Leg	1

TABLE 7. Poor responders

No. = 12	
Female 6, Male 6	
Colour	
Purple	0
Red	2
Pink	10
Site	
Face	10
Arm	0
Trunk	0
Leg	2

The details of the patients in each responding group are shown in Tables 4–7. In Table 8 the excellent and good responders are combined as the satisfactory responses and compared with the combined fair and poor responders (unsatisfactory).

Older age and purple coloured port wine stains are associated with a good response to treatment. Pink coloured port wine stains and a younger age do not respond as well to therapy. This trend is confirmed when the smaller groups of excellent versus poor responders are compared, with *P* values for age (< 0·003), purple (< 0·001) and pink port wine stains (< 0·001) all being highly significant.

Prior therapy was not significantly associated with a good or poor outcome from therapy. No patient with a purple port wine stain had a poor outcome from therapy, and 90% experienced a good or excellent result.

DISCUSSION

In this study of 100 patients, the continuous wave dye laser produced a good or excellent result in 63%, with a further 17% obtaining some benefit from treatment. Atrophic scarring, which in some patients consisted of a mild textural change with a shiny appearance and wrinkling, occurred in 14% and hypertrophic scarring in 5%. These results are comparable with those

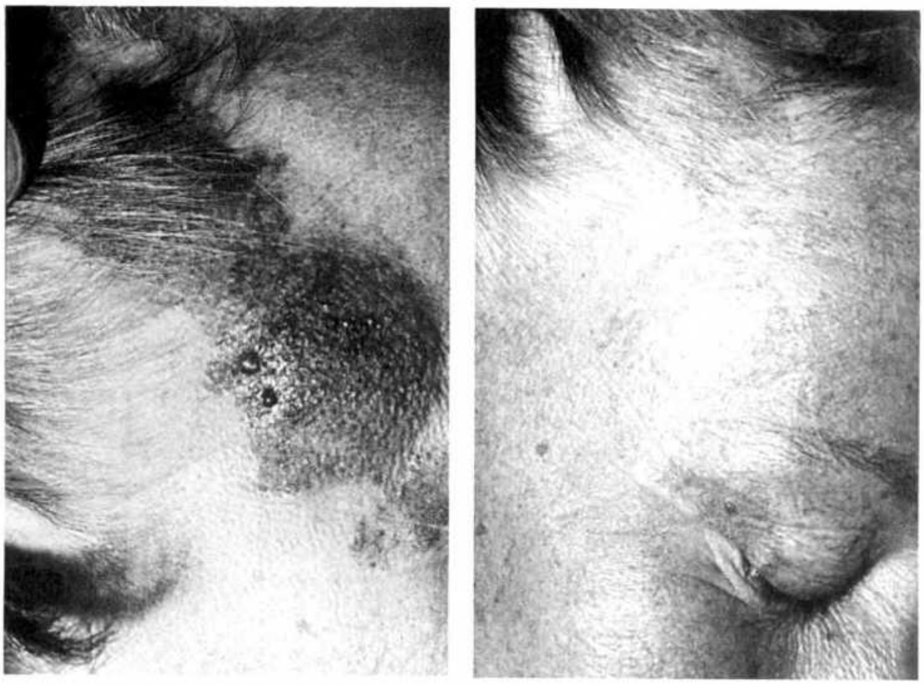


FIGURE 1. (a) Purple port wine stain on right temple extending into hairline. (b) Excellent result after laser treatment (the eyelid was not treated).

TABLE 8. Satisfactory (excellent + good responders, no. = 63) vs. unsatisfactory results (fair + poor responders no. = 29)

	Satisfactory	Unsatisfactory
Female	49	15
Male	14	14
Mean age (years)*	31·4	22·6
Colour		
Purple*	44	5
Red	11	6
Pink*	8	18
Site		
Face	59	24
Neck	2	1
Arm	2	1
Leg		3

* Comparison of adjacent groups $P = < 0{\cdot}001$.

published for the argon laser in the treatment of port wine stains.[3,4,11] In particular, the fact that better results are obtained in older patients with purple port wine stains is similar to results with the argon laser.

The histological effects of the argon laser on port wine stains have been studied[12,13] and

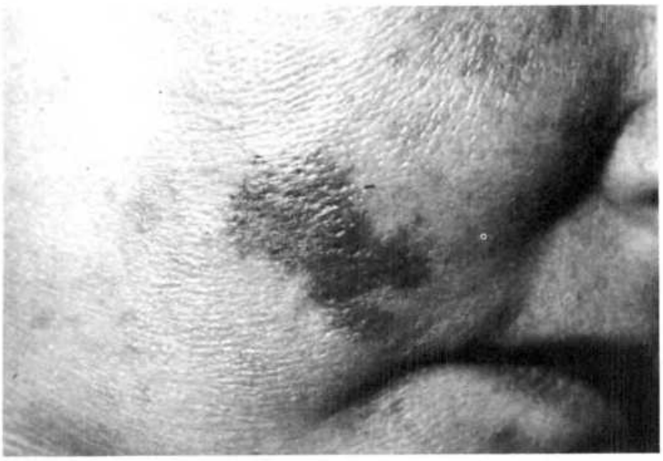
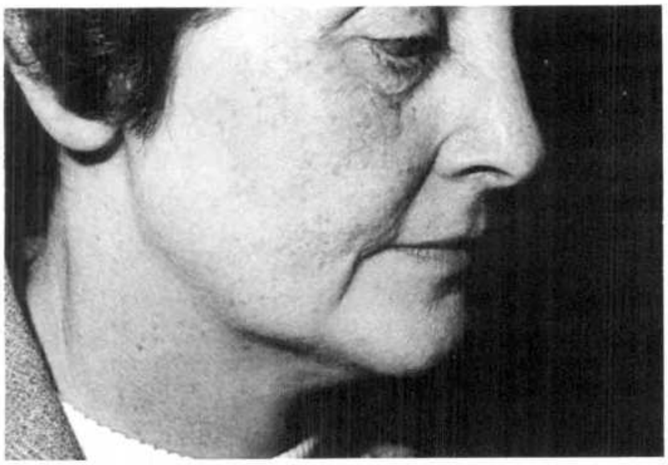


FIGURE 2. (a) Small port wine stain on the cheek.



(b) Excellent result after laser treatment.

compared with those following therapy with the pulsed tunable dye laser.[14] Following argon laser therapy, there is a non-specific epidermal and upper dermal coagulative necrosis. The pulsed tunable dye laser emitting at 577 nm produces erythrocyte aggregation and rupture of blood vessels, and an acute vasculitis with haemorrhage follows 48 h after treatment. There is very little damage to the epidermis and collagen and skin appendages are preserved. The visible appearance after pulsed dye laser therapy is a greyish-blue discoloration, as opposed to a blanching seen with the argon and continuous wave dye laser.

The similarity of the results from the continuous wave dye laser and the argon laser suggest that there is some non-specificity of tissue damage. To confine thermal damage within a target structure, energy must be delivered in less time than that required for cooling the target. For the ectatic vessels within port wine stains, this time—the thermal relaxation time—has been estimated to be 200–300 μs.[15,16] Thus, the pulse durations used in this study would inevitably result in non-selective thermal damage. The capabilities of the argon pumped laser used in this

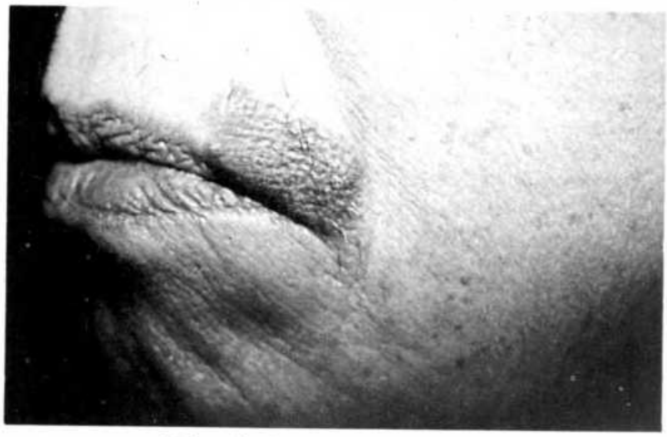
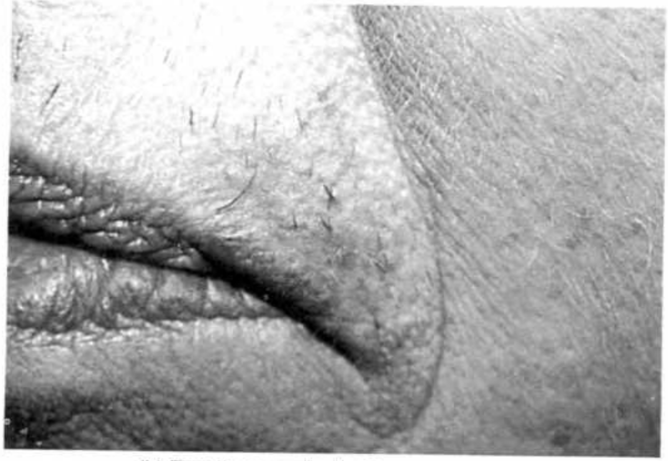


FIGURE 3. (a) Localized port wine stain on lateral upper lip.



(b) Excellent result after laser treatment.

study would have made it impossible to use microsecond pulse durations had they been required. It is not necessary to have selective thermal damage to blood vessels to improve the appearance of port wine stains as CO_2 laser treatment may be used[5,6] but theoretically there is an increased risk of scarring.

The results from pulsed dye laser therapy of port wine stains are still somewhat limited.[8,9,17] Satisfactory results have occurred in 73% of patients with a low incidence of scarring and pigmentary disturbances. The results from this study suggest that the continuous wave dye laser is an effective therapy for port wine stains in about two-thirds of the patients. There appears to be no advantage over argon laser therapy but those who fail to respond may then benefit from dye laser treatment. Patients with purple port wine stains will experience good results (90% of 49 patients with purple port wine stains had an excellent or good result), which is in contrast to experience with the pulsed dye laser to date. These differences are due to the pulse durations used and the use of minimal blanching power as an end point of treatment.

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A double-blind trial of treatment of seborrhoeic dermatitis with 2% ketoconazole cream compared with 1% hydrocortisone cream

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SUMMARY

Fifty patients with seborrhoeic dermatitis were treated with 2% ketoconazole cream ($n=24$) or with 1% hydrocortisone cream ($n=26$) for 4 weeks in a double-blind comparative study. These twice daily applications resulted in 87·2% symptomatic improvement for hydrocortisone vs. 81·6% for ketoconazole. The number of *P. ovale* yeasts was significantly reduced after the application of ketoconazole compared with hydrocortisone. The incidence of side-effects was low in both groups.

The role of *Pityrosporum ovale* in the aetiology of seborrhoeic dermatitis has been shown in a number of studies, and this condition has responded well to various topical or systemic antifungal agents.[1–4]

Ketoconazole is an imidazole derivative and has a broad spectrum as an antifungal agent. It is active against pityrosporum yeasts both *in vitro*[5] and *in vivo*.[6,7] Oral ketoconazole has produced hepatotoxicity[8,9] and interferes with the metabolism of testosterone.[10] Topical ketoconazole formulations have been found to be effective in controlling seborrhoeic dermatitis[11] without producing significant adverse reactions.

Several of the weak topical steroids are often used in the treatment of seborrhoeic dermatitis. As this condition is a chronic relapsing disease, the safety of these topical steroid preparations over a long period of time is questionable. This double-blind study compared the efficacy and safety of 2% ketoconazole cream with a standard therapy of 1% hydrocortisone cream.

METHODS

Fifty patients with seborrhoeic dermatitis were recruited for this study. The patients were examined at eight sites and graded numerically at each site into four categories on a 0 to 3 scale

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with 0 = clear, 1 = mild, 2 = moderate and 3 = severe. The eight sites included scalp, hairline, eyebrows, bridge of nose, nasolabial folds, external auditory meatus, posterior aspect of ear and sternum. The four categories included erythema, scaling, papules and pruritus. The maximum score obtainable at each visit was 96 (3 score × 4 different categories at 8 sites). Swabs were obtained for assessment of *P. ovale* from these sites. The patients were assigned tubes of either 2% ketoconazole cream or of 1% hydrocortisone cream in a randomized fashion. They were instructed to apply the cream to the scalp, face, sternum and ears twice a day. No other topical or oral medication considered as therapy for seborrhoeic dermatitis or affecting *P. ovale* was allowed, for the duration of the study. The patients were evaluated after 2 weeks of treatment. At each visit the eight sites were graded numerically for erythema, scaling, papules and pruritus. Swabs were also obtained for evaluation of *P. ovale*. The subjects received a global evaluation at the end of treatment. Global evaluations included total improvement (95–100% improvement), good (75–95% improvement), fair (50–75% improvement) and poor (less than 50% improvement).

Surface *P. ovale* colonization assessments were carried out as follows. If the number of colonies of *P. ovales* was above 20, colonization was numerous; if the number of colonies was between five and 20 it was intermediate; if there were less than five colonies it was sparse; and there was a final category of no growth.

RESULTS

Marked clinical improvement was observed in both treatment groups without significant differences between ketoconazole and hydrocortisone for scaling, itching, redness or for the total score of all symptoms. All the involved sites healed to almost the same extent in both of the treatment groups.

The percentage of symptomatic improvement was calculated from the total symptom score. There was no significant difference between the groups: ketoconazole cream application resulted in 81·6% symptomatic improvement while the application of hydrocortisone cream resulted in 87·2% symptomatic improvement (Fig. 1).

Although not significant, there was a difference in the global assessments. Of the 24 patients treated with ketoconazole cream 15 became totally clear (95–100% improvement), five were categorized as good (75–95% improvement), one as fair (50–75 % improvement) and three as poor (less than 50% improvement). Overall there were 87·5% responders in the ketoconazole group (Table 1). The 26 patients who were treated with hydrocortisone were evaluated as 19 totally cleared, six good and one deteriorated (96·2% responders—Table 1).

There was an obvious reduction of yeast cells in both treatment groups. This reduction was significantly higher for the ketoconazole treatment group (Mann–Whitney *U*-test, $P < 0{\cdot}05$) (Fig. 2).

In each group, one patient reported dry skin as an adverse effect of treatment. In the hydrocortisone group, another patient reported burning skin sensation.

DISCUSSION

This study has demonstrated that 2% ketoconazole cream is almost as effective as 1% hydrocortisone cream in the treatment of seborrhoeic dermatitis. Other studies have demonstrated that treatment with topical ketoconazole or oral ketoconazole is effective in seborrhoeic dermatitis. Ford *et al.*[3] reported the beneficial effect of 200 mg oral ketoconazole in a double-blind

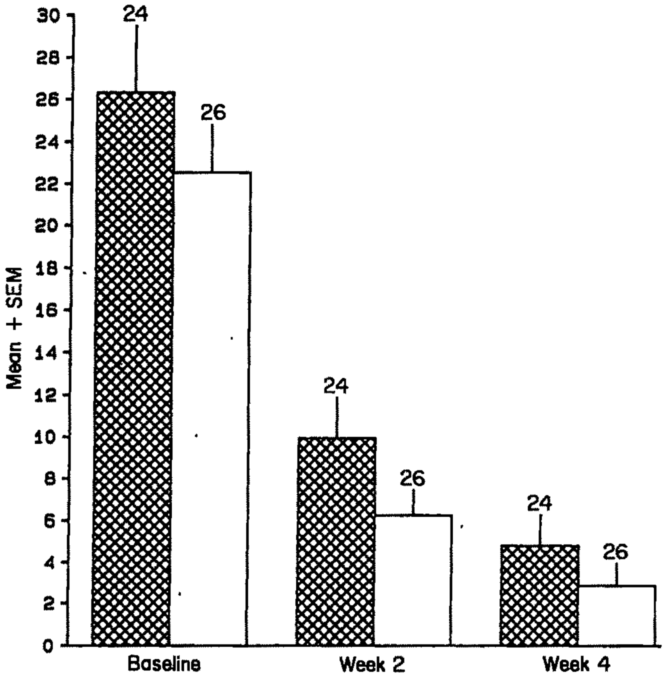

FIGURE 1. Evolution of the total symptom score during treatment with ketoconazole 2% cream ($n = 24$) or hydrocortisone 1% cream ($n = 26$). ▩, ketoconazole; □, hydrocortisone

placebo-controlled study. They concluded that a *Pityrosporum* yeast infection is the immediate cause of seborrhoeic dermatitis and that dandruff is a mild manifestation of the disorder. The removal of *P. ovale* with oral ketoconazole was found to lead to a clinical improvement of seborrhoeic dermatitis of the scalp,[12] and these results were obtained in patients with AIDS.[13] Two studies have reported a good response using 2% ketoconazole cream as compared with placebo.[14,15] Also good results have been obtained with topical ketoconazole in several uncontrolled studies.[16–18]

The decrease in *P. ovale* after treatment with ketoconazole cream together with the symptomatic improvement found in our study, supports the hypothesis that the yeast *P. ovale* plays an important role in the pathogenesis of seborrhoeic dermatitis.

TABLE 1. Global evaluation by the investigator at the end of treatment

	No. of patients			
	Hydrocortisone		Ketoconazole	
Cure	19	96·2%	15	87·5%
Good	6		5	
Fair	0		1	
Poor	0		3	
Deteriorated	1		0	

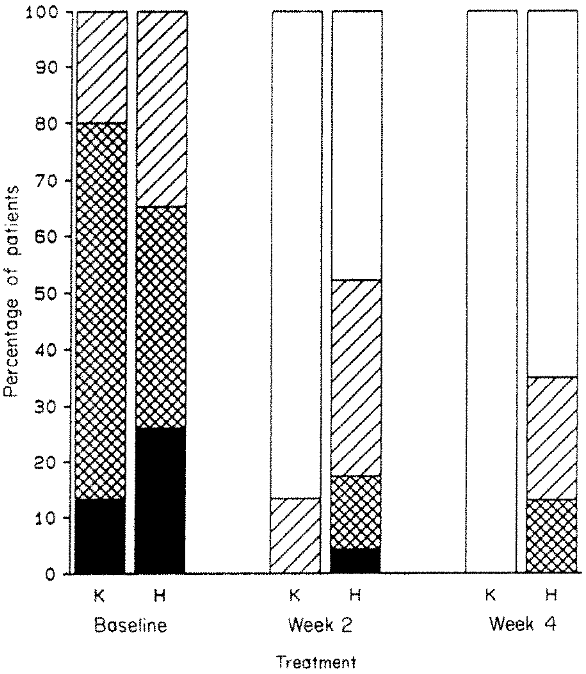

FIGURE 2. Changes in the number of *P. ovale* yeasts during treatment with ketoconazole 2% cream ($n = 24$) or hydrocortisone 1% cream ($n = 26$). ■, numerous; ▩, sparse; ▨, intermediate; □, absent.

In this clinical trial, more than 85% of the patients of the ketoconazole group responded to treatment. Although the rate of response was higher in the hydrocortisone group, ketoconazole cream appeared to be an effective non-steroidal alternative. The incidence of adverse effects was low in both groups but topical ketoconazole would be more suitable for long-term therapy. It would be of interest to study the possible additional benefit of using 1% hydrocortisone to reduce inflammation during the first week of treatment or using it in combination with ketoconazole cream.

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The effect of combined topical steroids and habit-reversal treatment in patients with atopic dermatitis

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SUMMARY

A modified behavioural method called habit-reversal, in combination with potent and weak corticosteroid cream, was compared with the use of the creams alone in the treatment of 45 patients with atopic dermatitis. The patients were randomly assigned to four groups, which received two different cream regimes in combination with the habit-reversal treatment. The patients' skin was assessed before, during and after treatment, and they recorded the amount of scratching during the study. The skin condition improved in all groups, but to a significantly greater degree in the habit-reversal groups. A strong correlation was found between the reduction in scratching and the improvement in skin status.

Atopic eczema often persists over a number of years though there may be periods of complete remission. The skin is often dry and irritable and this leads to scratching which aggrevates the eczema. Thus, a vicious circle is established. Areas that are easy to scratch and rub, for example the face, neck and hands, are more susceptible to chronic eczema. It has been suggested that scratching alone can cause eczema and lichenification of normal skin,[1] and it has been found that in patients with atopic dermatitis the histological changes occurred after the lesions had developed.[2]

Previous studies on scratching and dermatological skin disease have been carried out on only a few patients from a behavioural standpoint.[3–9] All these studies except one[9] were based on the idea of teaching the patients to ignore the itch. Several have found that habit-reversal procedures give good results,[8–11] the patients being taught to pat or strike the itchy areas instead of scratching. Cole *et al.*[12] reported a study in which a combination of a group approach and a behavioural self-control method was used. The scratching frequencies were not mentioned in this study, which makes the results difficult to interpret. The present study was designed to test the effect of modified habit-reversal (HR) treatment in combination with different cream

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regimes in patients with atopic dermatitis. The correlation between the skin status and the amount of scratching was investigated.

METHODS

Subjects

Forty-five patients (16 males, 29 females) with atopic dermatitis participated in the study. They were between 16 and 46 years of age (mean age 24·8 years) and were recruited from patients attending a private dermatological clinic. Two patients dropped out during the treatment period.

To be included in the study the patients had to be more than 16 years old, have no serious psychiatric disease and have moderate to severe, but not infected eczema.

Procedure

All were seen individually by a dermatologist, who explained the aim of the study and obtained their consent to participate. At the first visit they received instructions about recording the daily use of creams and the frequency of scratching, for which they were given a hand counter. A special record form was used which was for each day of the 5-week study. All assessments and the teaching of the habit-breaking method were performed by the dermatologist. A randomized four group design with three repeated measurements over time was used. (Fig. 1).

Measurements

Medical assessment. The skin condition of each patient was assessed with regard to four variables: dryness, scaling, erythema and infiltration. Each was graded on a 0–3 scale (0 = nil, 1 = mild, 2 = moderate and 3 = severe).

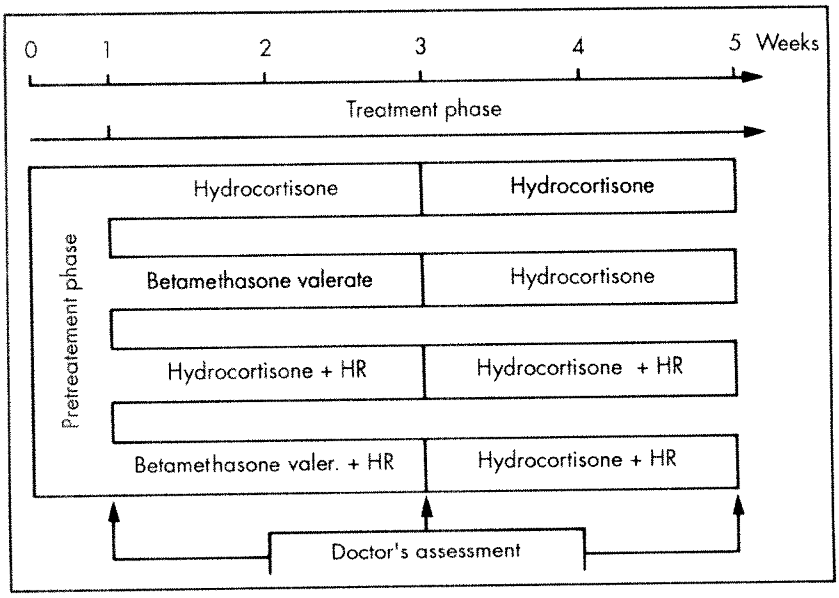

FIGURE 1. Design of the study, showing the four different groups, two treated with cream alone and two treated with cream together with habit-reversal therapy (HR).

Scratching. The frequency of scratching using a hand counter was recorded daily over a period of 5 weeks. The results were written down on the record form.

Compliance with the cream regimes. All patients were instructed to make a note on the form every morning and evening when the topical steroids were applied, and each time that the moisturizing creams were used.

Medical treatment. Groups 1 and 3 used a hydrocortisone (H) cream for the whole of the treatment period. Groups 2 and 4 used betamethasone (B) valerate for the first 2 weeks and a hydrocortisone preparation for the last 2 weeks. The steroid creams were applied twice daily. All patients were given the same instructions as to how to apply the creams.

Behavioural treatment. Groups 3 and 4 received the psychological treatment at the second visit which was briefly repeated at the third visit. This treatment was given by the dermatologist and consisted of a technique that was originally developed by Azrin and Nunn[10] for patients with nervous habits and tics, and as modified by Melin *et al.*[11] for patients with chronic atopic skin disease. The latter authors taught the patients in their study not to touch the skin when it was itching, but instead to clench their fists.

The patients in our study described and demonstrated how and when they scratched. The scratching behaviour was divided into two parts, the movement towards the itching area and the actual scratching. The patients were then taught two procedures, which are incompatible with the above movements, namely clenching their fists for 30 s and then pressing a finger nail or pinching the itching spot. Awareness was achieved by counting scratching episodes and practising the habit-reversal method several times in the doctor's office. The patients were also told to practise the method at least twice a day. If the itch vanished after 30 s of fist clenching the patients were told not to pinch the formerly itching spot.

Groups 1, 2, 3 and 4 are designated as follows: Group 1, hydrocortisone only (H); Group 2, betamethasone valerate and hydrocortisone (B + H); Group 3, hydrocortisone with habit-reversal therapy (H + HR); Group 4, betamethasone valerate and hydrocortisone with habit-reversal therapy ((B + H) + HR).

RESULTS

Total skin status

The total improvement in skin status scores in the four groups are shown in Figure 2. A split-plot ANOVA analysis of variance showed a significant time x group interaction ($F(6:82) = 4.2$ ($P < 0.01$)) indicating that the four groups changed differently. Subsequent analyses performed with Tukey's HSD test between the groups at different time periods showed no group differences before treatment, but after 2 and 4 weeks of treatment such differences were observed. After 2 weeks of treatment there were significant differences ($P < 0.05$) between groups: (B + H) and (B + H) + HR; H and (B + H) + HR; H and H + HR; and H and (B + H). After 4 weeks of treatment there were significant differences ($P < 0.01$) between groups: (B + H) and (B + H) + HR; H and (B + H) + HR; H and H + HR; and (B + H) and H + HR.

Separate variables of skin status

Analyses with comparisons of single effects were performed with Scheffe's test. Each of the four

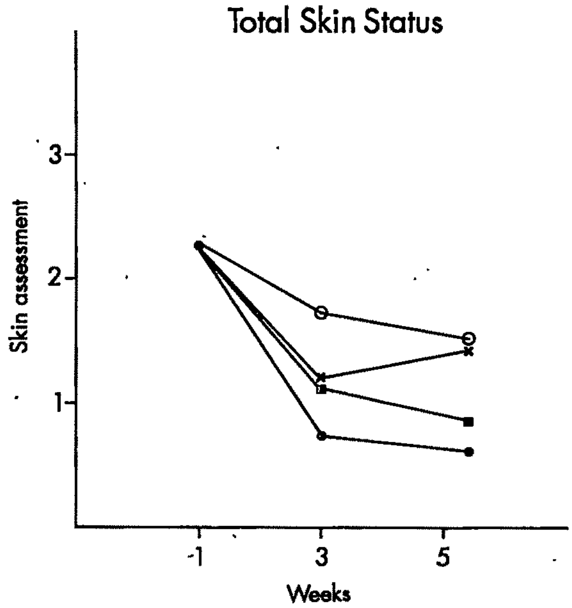

FIGURE 2. Total improvement in skin status scores in the four groups. Group H, 11 patients; B+H, 11 patients; H+HR, 13 patients; (B+H)+HR, 10 patients. O=H; ×=B+H; ■=H+HR; ●=(B+H)+HR.

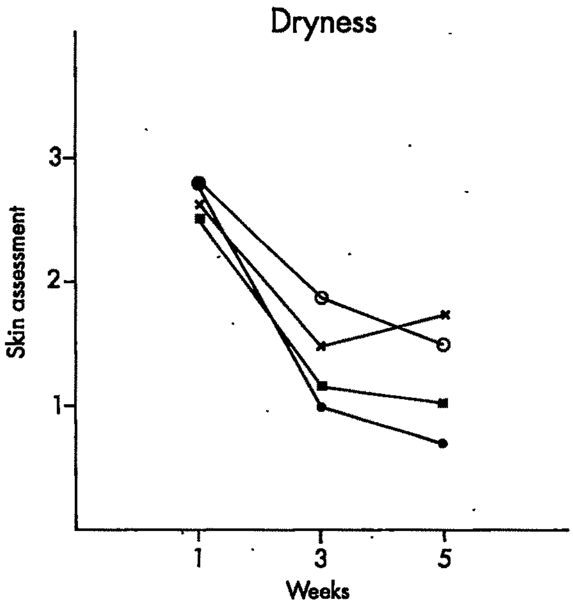

FIGURE 3. Changes in dryness scores in the four groups. The numbers of patients in each group are given in Figure 2. The difference between groups B+H and (B+H)+HR after 4 weeks of treatment is significant ($P < 0{\cdot}05$). There were no other significant differences. For key see Figure 2.

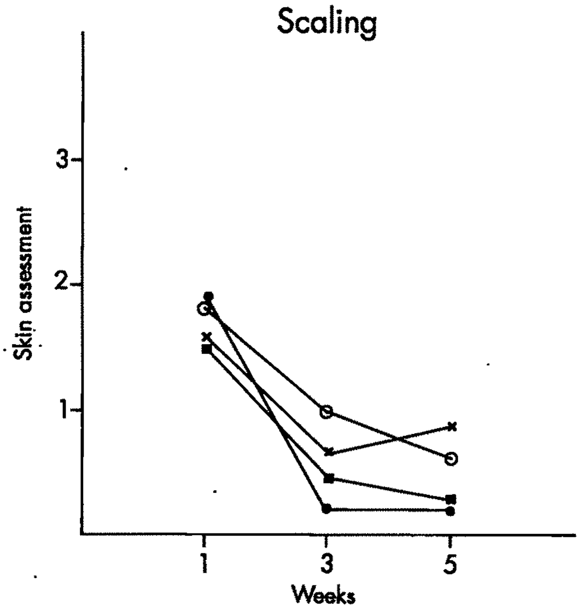

FIGURE 4. Changes in scaling scores in the four groups. The number of patients in each group is given in Figure 2. There are no significant differences between the groups. For key see Figure 2.

skin-status variables was analysed as a pre-post gains score. The gain scores for the different groups were compared by one-way analyses of variance.

Dryness. The dryness scores for the four groups are presented in Figure 3. ANOVA showed a significant time x group interaction among the groups ($F(3:40) = 3{\cdot}34$ ($P < 0{\cdot}05$)). Subsequent analyses with Scheffe's test showed that there were only differences ($P < 0{\cdot}05$) between groups (B+H) and (B+H)+HR.

Scaling. The scaling scores are shown in Figure 4. At ANOVA a non-significant time x group interaction was observed among the groups ($F(3:40) = 0{\cdot}3$).

Erythema. The erythema scores for the four groups are given in Figure 5. ANOVA showed a non-significant time x group interaction among the groups ($F(3:40) = 0{\cdot}2$).

Infiltration. The infiltration scores are presented in Figure 6. ANOVA showed a significant time x group interaction among the groups ($F(3:40 = 6{\cdot}0$ ($P < 0{\cdot}01$)). Subsequent analyses with Scheffe's test showed that there were differences between groups H and H+R; H and (B+H)+HR; and (B+H) and (B+H)+HR. The percentage reduction in scratching in the four groups can be seen in Table 1. ANOVA showed a significant time x group interaction among the groups ($F(3:41) = 9{\cdot}0$ ($P < 0{\cdot}001$)). Analyses with Scheffe's test revealed significant differences between groups: H and H+HR; H and (B+H)+HR; (B+H) and H+HR; and (B+H) and (B+H)+HR ($P < 0{\cdot}05$). The correlation between percentage improvement in scratching and improvement in skin status in the four groups combined was calculated and found to be 0·57.

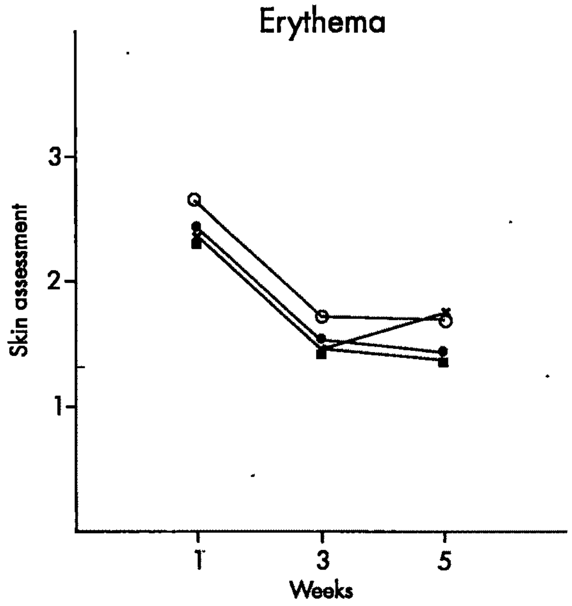

FIGURE 5. Changes in the erythema scores in the four groups. The number of patients in each group is given in Figure 2. There are no significant differences between the groups. For key see Figure 2.

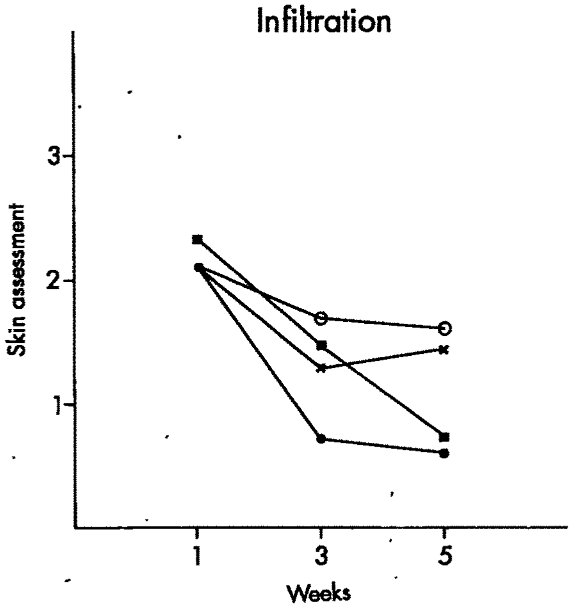

FIGURE 6. Changes in the infiltration scores in the four groups. The number of patients in each group is given in Figure 2. The differences between groups H and H+HR, H and (B+H)+HR, and B+H and (B+H)+HR are significant ($P<0{\cdot}05$). For key see Figure 2.

TABLE 1. Percentage reduction in scratching

Group	No. of patients	Per cent
H	11	65
B+H	11	74
H+HR	13	88
(B+H)+HR	10	90

DISCUSSION

The results of this study clearly show that habit-reversal is an effective method of reducing scratching and thereby improving the skin status in patients with atopic skin disease. The results also indicate that the best treatment seems to be a combination of habit-reversal and a potent topical steroid. This has been shown in other studies [8–11] where the percentage reduction in scratching was 79 and 75% respectively. In the present study the reduction was 88% in group H+HR and 90% in group (B+H)+HR. In group H there was a reduction of 65% and in group B+H, 74%. The reason for the very high reductions in groups H+HR and (B+H)+HR is evidently that these patients were able to influence the itch without damaging the skin. The results in groups H and B+H are probably due to the consistent use of both steroids and emollients. We know from other studies[3,13,14] that attention may reinforce and maintain scratching behaviour.

The two variables of dryness and scaling correlated with the use of steroids and emollients and showed little or no difference between the groups which all improved. Erythema and especially infiltration were the two variables that correlated well with the mechanical irritation of the skin. The greatest changes in these signs were noted in the groups receiving habit-reversal therapy.

Group B+H differed from the rest in that the condition deteriorated during the last 2 weeks of treatment. One explanation could be a combined effect of steroid rebound and inefficient healing. This was not seen in group (B+H)+HR where the healing had advanced further and this supports the habit-reversal method. There was a highly significant correlation between skin status and scratching, which meant that about 50% of the healing could be accounted for by a reduction in scratching.

The dermatologist gave the psychological treatment as well as performing the assessment, a procedure which is open to criticism, but there was a good correlation between the reduction in scratching as monitored by the patients and the clinical evaluation in the groups. Furthermore, the dermatologist was not aware of which steroid was used during the first 2 weeks.

This study clearly demonstrates the importance of teaching patients to control their scratching. No long-term follow-up was carried out and therefore the relapse rate cannot be determined. However, this must be relatively high and further studies are indicated for the long-term management of these patients to determine the relapse rate.

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Isotretinoin in the treatment of systemic sclerosis

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SUMMARY

Thirteen patients with systemic sclerosis were treated with isotretinoin. Nine patients completed between 6 and 14 months of treatment and all showed an improvement in the cutaneous manifestations of their disease. The drug did not appear to benefit internal organs affected by the disease. Most patients experienced the well-recognized side-effects of retinoids, which in three cases necessitated withdrawal from the study within 3 months. Serum levels of type III procollagen aminopropeptide did not show a consistent decline during treatment, despite a clinical improvement. The mode of action of the reported therapeutic effect of isotretinoin in systemic sclerosis is unclear. There may be a preferential suppression of the synthesis of type I collagen, or the drug may be acting by an unrelated mechanism.

Systemic sclerosis (SS) is a progressive, disabling disorder, for which there is little effective treatment. The vitamin A derivative, isotretinoin, has an established therapeutic role in several dermatological disorders and recent *in vitro* work with this drug has indicated that it has the capacity to inhibit collagen synthesis by fibroblasts. This appeared to be a good theoretical indication for examining its effect in patients with SS, and we have performed an open study of the effect of this drug in a group of patients with this disease.

METHODS

Thirteen patients (two male, 11 female; mean age 53 years, age range 20 to 71 years) with SS, who fulfilled the American Rheumatism Association diagnostic criteria,[1] were included in the study. Four patients had already received treatment for their SS in the form of systemic corticosteroids, penicillamine or colchicine, but in no case had this prevented progression of the disease. One patient remained on a constant dose of prednisolone (7 mg daily) and penicillamine (250 mg daily) throughout the study. No other patients were taking any medication that could have influenced the course of their disease. Details of the patients' disease and of previous treatments are given in Table 1.

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All patients gave informed consent to participating in the study and females of child-bearing potential signed an additional form undertaking not to conceive during the period of treatment with isotretinoin and for 3 months afterwards. The study protocol had been granted ethical approval.

Prior to treatment, the following investigations were performed: full blood count and differential, ESR, biochemical screen including fasting lipids and creatine kinase, creatinine clearance, autoantibodies, immune complexes, C3 and C4 levels, immunoglobulins, protein electrophoresis, urinalysis, chest radiography and lung function tests. Patients were given isotretinoin at a dose of 1 mg/kg body weight daily. A full clinical examination was done before and after treatment, and at 6 months in those patients who received treatment for longer than this period. The extent and severity of the cutaneous sclerosis was expressed in terms of a 'skin score'. This score was assessed at each of 16 body sites on each side of the body on a 0–3 scale, where a score of 0 represented normal, 1 doubtfully involved, 2 definitely involved and 3 grossly involved skin. The skin sites were as follows: upper face, lower face, neck, anterior chest, abdomen, upper back, lower back, fingers, hands, forearms, upper arms, shoulders, thighs, legs, feet, and toes. The totals for the two sides of the body were summed, giving a maximum possible skin score of 96. A full range of clinical photographs was also taken pre- and post-treatment. During the study, patients were examined at monthly intervals by the same physician and any changes noted by the patients, including those representing possible side-effects of treatment, were recorded.

In order to monitor for side-effects, fasting lipids, urea and electrolytes, liver function tests and blood count were checked monthly for the duration of treatment. At the end of the treatment period all the investigations performed on entering the study were repeated.

Measurement of type III collagen aminopropeptide

Serum samples were collected from a group of ten patients with SS and from 12 age-matched controls and stored at −20°C until ready for analysis. In seven of the patients treated with isotretinoin, serum samples were taken prior to commencing the drug and at regular intervals during treatment, and were stored in the same way. The measurement of type III collagen aminopropeptide (PIIIP) was performed using a commercially available radioimmunoassay kit, manufactured by Berhringwerke AG (Marburg, Germany) and purchased from Hoechst AG (Hounslow, U.K.). The Fab assay, which uses the Fab fragments of the PIIIP antibody instead of the intact IgG, was employed. Each serum was assayed in duplicate and all assays were carried out on one occasion using the same kit.

Statistical analysis of the skin scores before and after treatment was performed using the paired *t*-test, and of the PIIIP levels in the group of SS patients and controls using the Student's *t*-test.

RESULTS

Details of treatment and response in each patient are given in Table 1.

Of the 13 patients entered into the study, nine completed between 6 and 14 months of treatment, while the remaining four received treatment for less than 14 weeks. Of the four who terminated treatment prematurely one, a 72-year-old female, died after 4 weeks from a deep-vein thrombosis and pulmonary embolism, which were thought to be unrelated to both her disease and the isotretinoin. The other three patients were withdrawn because of troublesome facial erythema or hypertriglyceridaemia. No improvement in the skin was seen in these four patients.

TABLE 1. Details of patients with systemic sclerosis included in the study.

Patient no.	Age	Sex	Disease duration (years)	Internal organs affected	Previous treatments	Isotretinoin (mg/kg per day)	Duration of treatment	Reason for stopping	Response
1	60	F	12	—	—	1	3 days	facial swelling and erythema	—
2	56	M	6	muscles, GIT* lungs, joints	prednisolone	1	2 weeks	increased TG†	—
3	71	F	2	lungs, joints	—	1	4 weeks	deceased	—
4	51	F	16	oesophagus	—	1	14 weeks	facial swelling and erythema	—
5	61	F	15	oesophagus lungs	—	1	6 months	increased TG	skin looser, finger-tips better
6	58	F	15	—	—	1	6 months	increased TG	decreased skin oedema paronychias better
7	55	F	25	lungs, joints	—	1	7 months	increased TG	skin looser
8	55	F	3	lungs, joints, GIT, Sjögren's	—	1	8 months	remission	skin looser Raynaud's better
9	49	F	4	lungs, joints	penicillamine	1	8 months	pleural effusion	skin softer decreased pigmentation
10	54	F	2	lungs, joints, GIT, Sjögren's	penicillamine prednisolone	1→0·6	1 year	peptic ulcer	skin softer finger-tips better
11	45	F	4	—	—	1	1 year	remission	skin looser Raynaud's better
12	20	F	7	lungs	—	1→0·3	14 months	no further benefit	skin looser decreased pigmentation
13	50	M	5	lungs, joints, GIT, muscles	colchicine penicillamine prednisolone	1	14 months to date	continuing	skin looser

* GIT = gastrointestinal tract.
† TG = triglycerides.

In the remaining nine patients, an improvement in the skin was observed in every case. This took the form of diminished sclerosis, tethering and oedema, with the patients describing the skin as becoming 'looser' of 'softer' and swelling of the face and hands diminishing. Other indications of an improvement were reports of performing manual tasks with greater ease. Patients found that they could grip more easily. In two patients, there was a reduction in the diffuse hyperpigmentation associated with their disease and in a further three, there was resolution of finger-tip fissuring and recurrent paronychias. Episodes of paronychia in one patient, however, continued while on isotretinoin. Raynaud's phenomenon remained unchanged in seven subjects and improved in only two. Symptoms relating to internal organ involvement by SS, such as dyspnoea on exertion, dysphagia and arthralgia failed to improve with isotretinoin.

The improvement noted by both patient and physician in the skin was borne out by the pre- and post-treatment skin scores, which diminished in all cases ($P < 0{\cdot}05$, Fig. 1). Examples of the clinical improvement seen in two patients are shown in Figures 2 and 3.

None of the laboratory parameters reflecting disease activity, in particular measurements of renal function, ESR, antinuclear antibodies and complement levels, showed any consistent change during treatment. Pulmonary function tests showed an overall deterioration in FEV_1 and K_{co}. Whether this can be attributed entirely to the disease or whether it was accelerated by the administration of isotretinoin is being investigated and will form the subject of a separate report.

Side-effects encountered in the nine patients on long-term treatment with isotretinoin included hypertriglyceridaemia in five cases, one of whom also developed hypercholesterolaemia. This necessitated eventual withdrawal from the study in three cases (the highest level of

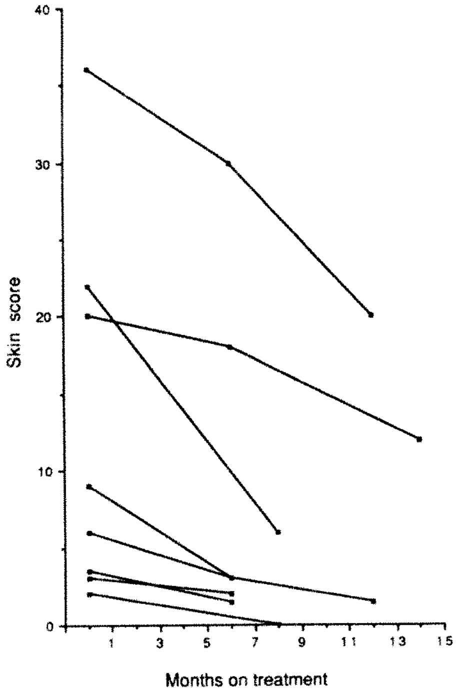

FIGURE 1. The effect of isotretinoin treatment on the skin scores in patients with systemic sclerosis.

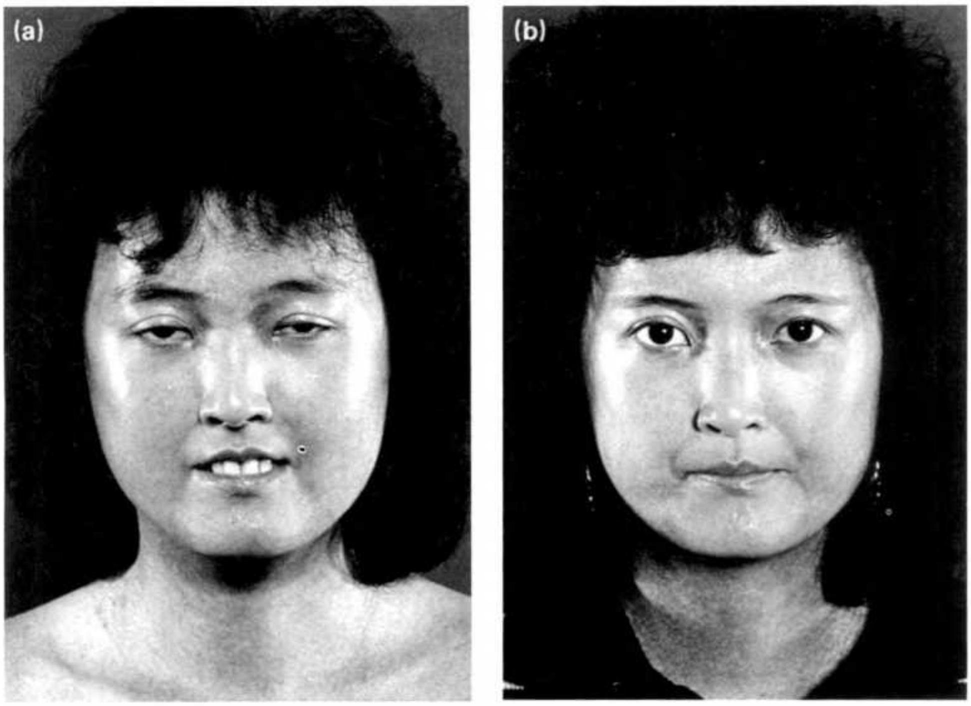

FIGURE 2. Patient's face (a) before and (b) after 11 months on isotretinoin, showing the reduction in swelling and sclerosis.

plasma triglyceride recorded being 3·9 mmol/l), but the other two responded satisfactorily to dietary measures, with reduction in the dose of isotretinoin in one of them. Most patients experienced the well-recognized mucocutaneous side-effects of the drug. One patient complained of alopecia and another noted that her hair had become curly. In one patient, there was a recurrence of a previously healed leg ulcer. Two patients complained of aching of the shoulder girdle and neck during the early weeks of treatment, but this resolved spontaneously despite continuing isotretinoin. One patient terminated treatment after 1 year because of increasing dyspepsia. Another was withdrawn from the study after 8 months because of an eosinophilic pleural effusion. The details of this case have been reported separately.[2]

Measurement of type III collagen aminopropeptide

This was measured in 10 untreated patients with SS (mean age 50 years; nine females, one male) and in 12 healthy controls (mean age 46 years; eight female, four male). The level of PIIIP (mean ± SD) was 54 ± 22 ng/ml in the patients with SS and 30 ± 8 ng/ml in the controls ($P < 0{\cdot}005$). Of the seven SS patients treated with isotretinoin in whom PIIIP levels were measured, none showed a decline in the levels during treatment, despite an improvement in their skin (Fig. 4).

DISCUSSION

Isotretinoin has been shown in this study to improve several of the cutaneous manifestations of SS. A good response was seen after between 6 months and 1 year of treatment. However, there was no evidence of a beneficial effect in internal organs affected by the disease. It is also not yet

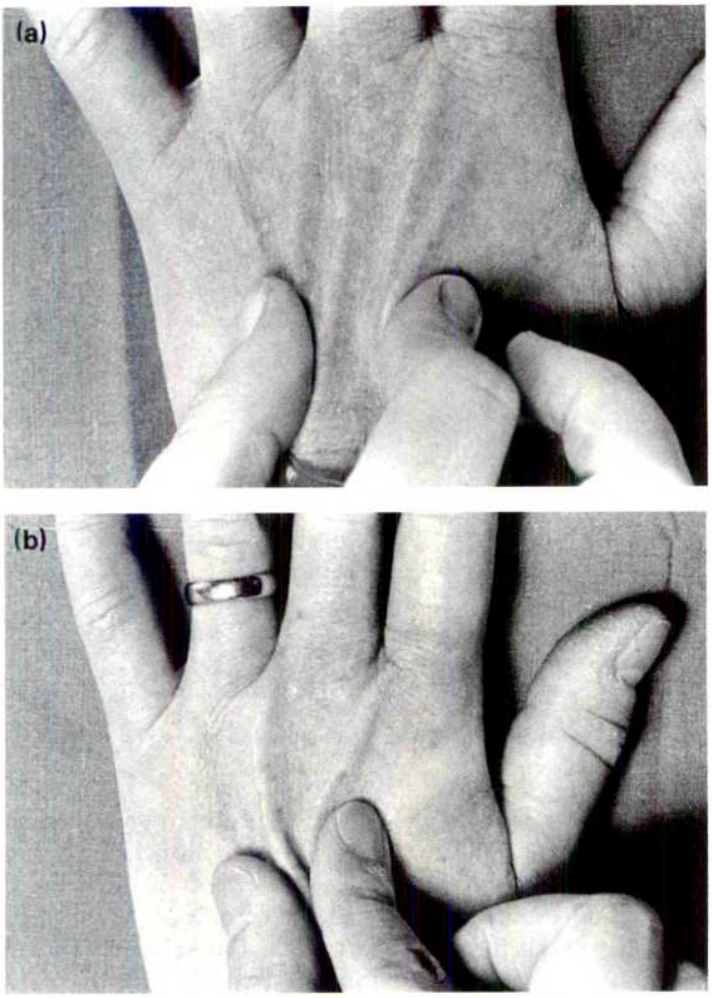

FIGURE 3. Skin on the dorsum of a patient's hand being wrinkled to the maximum extent possible. (a) before and (b) after 6 months on isotretinoin.

possible to say whether the remission achieved in the skin is long-lasting. The only other report of a beneficial effect of isotretinoin in SS was of three patients who experienced a subjective improvement after only a few weeks on a dose of 20–50 mg daily.[3] However, etretinate was found to be a useful treatment for SS in an open study of eight patients.[4] In a double-blind placebo-controlled study of tretinoin cream in the treatment of keloids and hypertrophic scars with measurement of lesion volume, a highly significant reduction in the tretinoin-treated group was demonstrated.[5]

Various drugs have been used for the treatment of SS. The agent for which there is best evidence of a therapeutic effect is probably penicillamine, although in the study of Steen *et al.*[6] at least 19 months of treatment was required. Others have found only a limited cutaneous improvement with penicillamine.[7] Colchicine was found to be effective in the treatment of SS in one study,[8] but not in another.[6] There is no evidence that steroids or other immunosuppressive agents retard the progress of the disease.

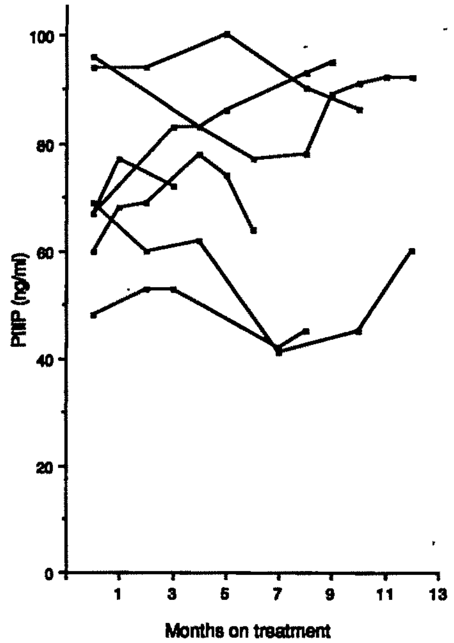

FIGURE 4. The effect of isotretinoin treatment on the serum levels of type III collagen aminopropeptide (PIIIP) in patients with systemic sclerosis.

In normal adult skin, types I and III collagen are major constituents of the dermis,[9] occurring in a ratio of approximately 4 to 1. Two *in vitro* studies[10,11] of collagen synthesis by cultured dermal fibroblasts from patients with SS have shown that this is increased, without any change in the type I/III procollagen ratio. Lovell *et al.*,[12] using immunofluorescence examination of normal and SS skin sections, also showed that the proportion of the two collagen types was unchanged in diseased skin.

Retinoids have been shown to inhibit the proliferation of normal human skin fibroblasts *in vitro* and the synthesis of both type I and type III collagen.[13] Ohta and Uitto[14] have demonstrated an inhibitory effect of isotretinoin on the levels of type I and type III procollagen messenger RNAs and on procollagen production by control and SS fibroblasts. There is therefore a sound theoretical basis for the therapeutic effect of isotretinoin in the skin of patients with SS. The therapeutic effect could not be explained in terms of increased collagen breakdown, because retinoids are not known to stimulate collagenase production and in fact some retinoids inhibit it.[15]

In an attempt to monitor the response to treatment in SS objectively, it was decided to measure sequentially the serum levels of PIIIP. PIIIP is the amino-terminal peptide released from type III procollagen during its conversion into collagen. The serum levels of PIIIP can be measured by radioimmunoassay and appear to be elevated in conditions where there is a high activity of ongoing fibrosis with inflammation, for example acute hepatitis and active fibrotic liver disease,[16] fibrosing alveolitis[17] and myelofibrosis.[18] Krieg *et al.*,[19] using the Fab assay, found that the mean PIIIP level was approximately 50% greater in a group of SS patients than in normal controls and that the levels correlated with disease severity. In the present study, an 80% increase was found in the SS patients.

If the therapeutic effect of isotretinoin in SS is due to inhibition of collagen synthesis, one might have expected a reduction in PIIIP levels with treatment, but this was not seen. It is however possible that the major effect of isotretinoin in SS is on the synthesis of type I collagen, which would not be reflected in the PIIIP assay. Further studies are required in which type I procollagen aminopropeptide is measured. Alternatively, isotretinoin could produce its therapeutic action through an effect on glycosaminoglycan synthesis, as suggested by a recent *in vitro* study.[20]

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Acitretin monotherapy in Darier's disease

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SUMMARY

The dose response relationship to acitretin in five patients with Darier's disease was investigated. The patients received in the first 3 months 35 mg daily, and the dose was adjusted according to the clinical response. Four of the five patients showed a marked improvement with up to total clearance, although in two patients isomorphic reactions due to a relative overdose were seen. These isomorphic reactions improved with a reduction of dose. We suggest that treatment with acitretin is initiated at 10–25 mg daily with gradual adjustment of the dose.

Darier's disease (DD) or keratosis follicularis is inherited as an autosomal dominant trait and is characterized by predominantly follicular pruritic hyperkeratotic papules. The onset is usually in late childhood and the course of the disease is chronic and in most cases slowly progressive.

Before oral retinoids became available, the response to different kinds of treatment was poor. The use of oral vitamin A was limited by toxicity and topical retinoic acid was usually unsuccessful due to local irritation.[1] Etretinate has been found to be effective in the treatment of DD,[2–6] but is difficult to find an optimal dosage schedule for this drug[2,3,5,6] and even a slight overdosage may result in an isomorphic reaction with consequent deterioration. Despite this, etretinate has become the first choice in the therapy of this condition.

The new oral retinoid acitretin (Ro 10-1670) has been found to be effective in psoriasis and various disorders of keratinization.[7–9] Acitretin is the free acid of etretinate and the main metabolite of etretinate. It has an elimination half-life of approximately 50 h, in contrast to that of etretinate of 80–160 days.

We investigated the dose–response relationship of five patients with DD during treatment with acitretin to determine the practical management of this disorder using this retinoid.

METHODS

Two women and three men with moderate to severe DD were treated with acitretin capsules (Hoffmann-La Roche, Basle, Switzerland). Female patients of child-bearing age were not included unless using reliable contraceptive methods, and patients with concomitant diseases, such as disturbances of the lipid metabolism, were also excluded. No additional therapy was allowed apart from emollients. Three patients were previously treated with etretinate and this was discontinued at least 4 weeks before the trial.

Before therapy and each month thereafter blood investigations were performed, including renal and liver function tests and the lipid levels. The patients were seen monthly and more frequently if necessary. The clinical results were scored using a 4-point scale for erythema, scaling, hyperkeratotic papules and pruritus (0 = not present; 1 = mild; 2 = moderate; 3 = severe). The total severity score refers to the sum of all 4-point scales. Photographs of each patient were taken several times during the trial, and at each visit the patients were asked about side-effects. Treatment with acitretin was continued for at least 6 months. The initial dosage was 35 mg per day and after 1 month the daily dose was adjusted according to efficacy and in relation to the side effects.

RESULTS

The clinical results are summarized in Figure 1.

Case 1

A 47-year-old woman had a history of slowly progressive DD for 20 years. The initial severity score was 9. Acitretin treatment was started at an initial dosage of 35 mg per day and after 1 month the erythema and the hyperkeratotic papules improved but her pruritus was the same. During the next 2 months she was treated with 25 mg acitretin per day, and the clinical picture did not change. Increasing the dosage to 35 mg per day resulted in an exacerbation of symptoms over the next 2 weeks, probably due to an isomorphic reaction. Subsequently the dosage was reduced to 10 mg per day with improvement, and on increasing to 20 mg per day there was a slight exacerbation. She has been on 10 mg acitretin per day for 7 months and remains improved without any exacerbations.

Case 2

This 41-year-old woman first developed symptoms of DD when she was 6 years old. The patient had a severity score of 11 and was initially treated with 35 mg acitretin per day with some improvement. Increasing the dose after 3 months to 60 mg per day produced a mild exacerbation with later a distinct improvement, although the disease continues to be very active.

Case 3

A male patient, 39 years of age, had severe DD since late childhood. The severity score prior to treatment was 12. The patient received 35 mg acitretin daily during the first 3 months, and a mild improvement was noticed. During the fourth month the dose was increased to 45 mg per day. His itching became worse and we decided to decrease the dosage to 25 mg per day. This resulted in a marked improvement, although a satisfactory result was not achieved.

Case 4

A 36-year-old male patient had DD of moderate severity. The first symptoms started at the age of 29. The initial severity score was 9. During the first 2 months the patient was treated with 35 mg acitretin daily. This resulted in a complete clearance but decreasing the dosage to 25 mg was followed by a slight exacerbation. On maintenance therapy with 35 mg acitretin the patient has remained free of symptoms.

Case 5

A 47-year-old male had a 10-year history of moderate DD. The initial severity score was 8, and

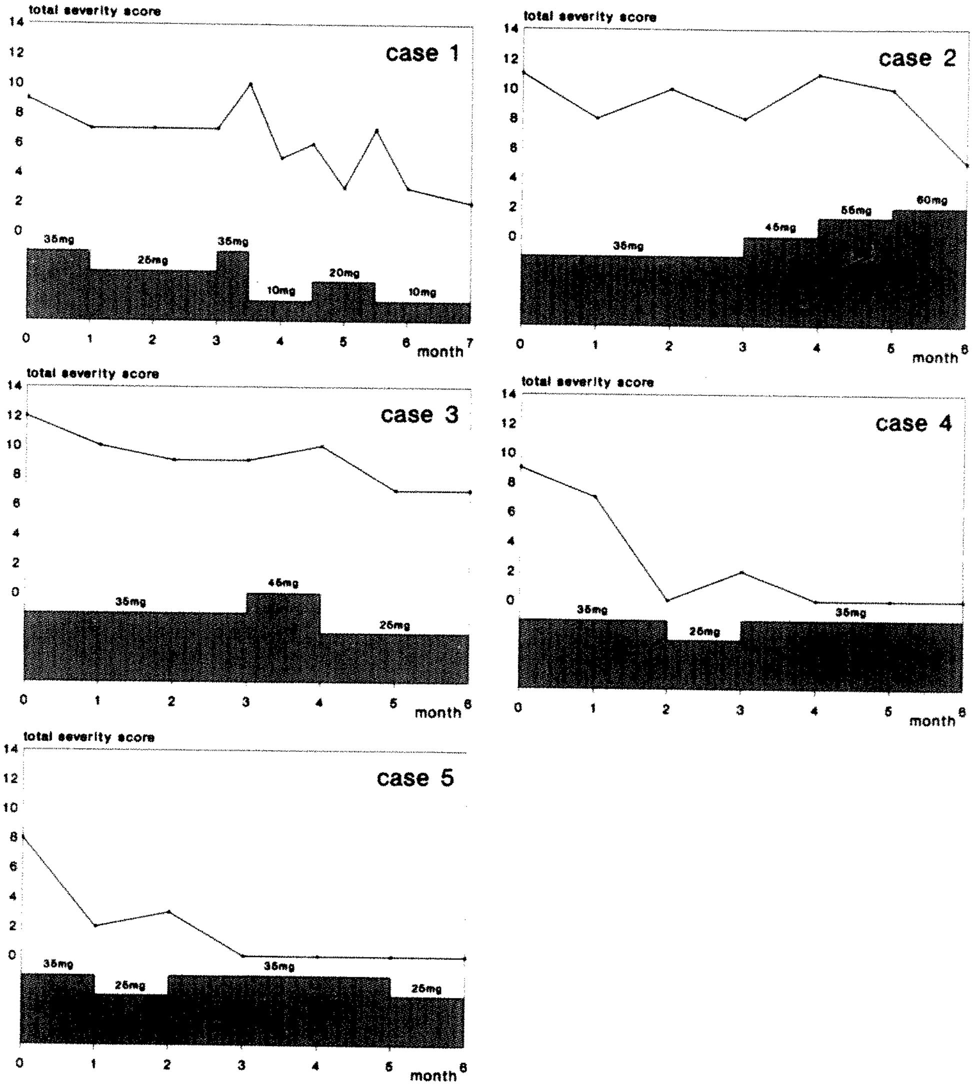

FIGURE 1. Total severity score in five cases of Darier's disease during treatment with acitretin. The scoring refers to the sum of erythema, scaling, hyperkeratotic papules and pruritus.

his initial dose of acitretin of 35 mg daily resulted after 1 month in a marked improvement. On 25 mg per day there was a slight exacerbation but increasing the dose to 35 mg resulted in total clearance after 3 months (Fig. 2). This clearance was maintained with a dosage of 25 mg per day.

Side-effects

Treatment with acitretin was well tolerated by all the patients. A mild to severe cheilitis was noticed by each patient and two of them complained of a dry mouth and one patient had a dry skin. In two cases there was a mild elevation of the serum triglycerides during the trial period.

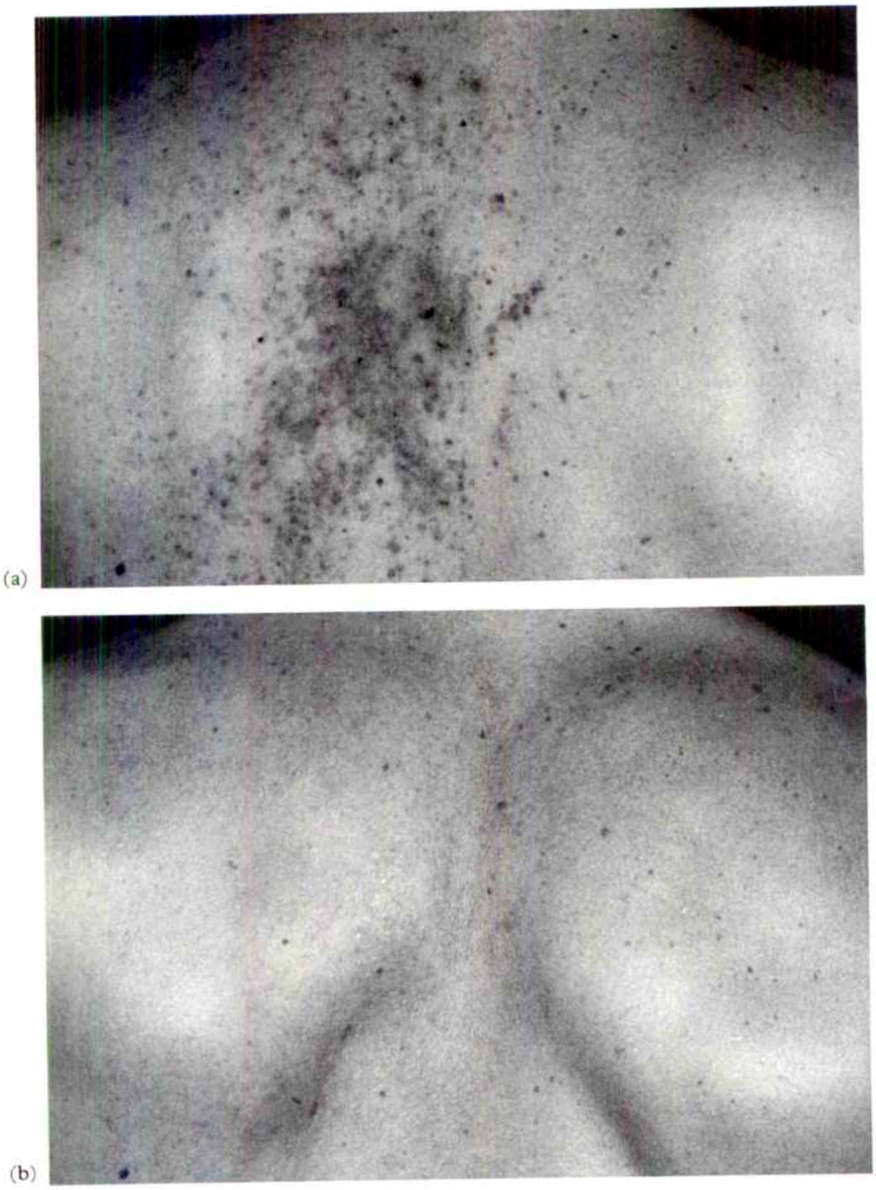

FIGURE 2. Darier's disease (Case 5) (a) before treatment; (b) after 5-month treatment with acitretin.

DISCUSSION

In this trial all the patients with DD improved during treatment with acitretin. In two there was complete clearance of the eruption and two showed a marked improvement. In the remaining case there was only a moderate improvement of the disease.

The dose of retinoids in the treatment of DD is a problem. The initial dosage of etretinate has been suggested as between 0·5–1·0 mg/kg,[2,3] but a lower dosage of 0·3–0·5 mg/kg has been advocated.[4–6,10] This is because severe isomorphic reactions have been reported with high dose etretinate therapy.[3–6] With acitretin an initial dose of 25–35 mg per day has been suggested for the treatment of various disorders of keratinization.[9] This study shows that the therapeutic safe range of acitretin in DD is limited and that isomorphic reactions can occur on relatively low doses. However maintenance therapy in four of our five patients with 10–35 mg per day proved to be effective, and this is similar to another study on DD[11] which showed that acitretin in a dose of 20 mg per day improved the majority of 13 patients. We consider that it is probably advisable to start with 10–25 mg acitretin per day and then to adjust the dose by gradual increments up to the optimal therapeutic level for the individual patient.

The relatively short half-life of acitretin compared to etretinate is a major advantage, particularly with regard to the period of strict contraceptive precautions that are necessary after stopping therapy. A second advantage is the prompt response with a reduction in the dose when side-effects have occurred.[12] This is important in those diseases where slight overdosing results in serious adverse reactions. Our study indicates that acitretin appears to be of value in the treatment of DD and superior to etretinate in the practical management of this disorder.

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Lymphomatoid papulosis: ultrastructural, immunohistochemical and gene analytical studies

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SUMMARY

Ultrastructual, immunohistochemical and gene analytical studies were carried out on a 39-year-old patient with lymphomatoid papulosis. Two different cell groups were demonstrated in the papulonodular eruptions: large atypical cells with multiple nuclei that were well stained with anti-Tac, but not with Leu 3a, and other cells that possessed prominent hyperchromatic nuclei and which stained well with Leu 1 and Leu 3a but not with anti-Tac. Gene analytical studies using *Eco*RI, *Bam*HI and *Hind*III revealed no rearrangement, indicating a non-clonal T-cell proliferation unlike malignant T-cell lymphoma. These results suggest that the present case was benign.

The term 'lymphomatoid papulosis' was first used by Macauley for a self-healing eruption that appeared to be histologically malignant but clinically benign.[1] Lymphomatoid papulosis is characterized by the appearance of papular lesions predominantly on the trunk, which become necrotic. The lesions often heal to leave a scar. The disease usually has a chronic course often lasting several years.[2–5] The histology of the lesions is consistent with that of a malignant lymphoma and shows a mononuclear cell infiltration in the dermis and epidermis. There are large atypical mononuclear cells that contain cerebriform nuclei and also cells resembling the Reed–Sternberg cells as seen in Hodgkin's disease.[4]

We describe a patient with lymphomatoid papulosis, and present ultrastructural, immunohistochemical findings and an analysis of the rearrangement of the T-cell receptor gene.

CASE REPORT

A 39-year-old man was admitted to hospital with a necrotic papulonodular cutaneous eruption over the whole body in January 1987. He first noticed the eruption on his trunk and extremities 5 months previously. Each lesion began as a small reddish papule and after enlarging developed

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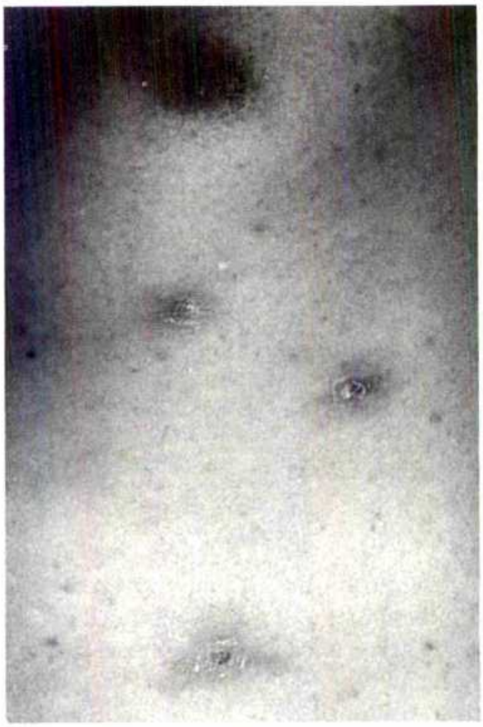

FIGURE 1. Red papules with black irregularly-shaped crusts on the anterior chest. Similar lesions were distributed on the extremities and trunk.

central necrosis with a crust formation and finally healed spontaneously within 4 weeks. He suffered neither pain nor itching.

Physical examination revealed multiple red, indurated papules and nodules on the trunk and limbs, some of which were covered with a dark irregular-shaped crust (Fig. 1). The diameter of each papule varied from 3 to 30 mm. The lesions cleared to leave brown areas of post-inflammatory hyperpigmentation. His general health was good and there was no lymphadenopathy and no hepatosplenomegaly.

The results of laboratory investigations were either within normal limits or negative. These included complete blood cell counts, erythrocyte sedimentation rate, blood chemistry studies, protein electrophoresis, immunoglobulin levels, lipid levels, antinuclear antibody, serum electrolytes, urinalysis, antibodies against HTLV-1 and HIV, ^{67}Ga-scintigraphy, and chest X-ray. The Mantoux reaction was positive and mitogenic stimulation with phytohaemagglutinin or concanavalin A of peripheral blood lymphocytes was normal.

The histology of necrotic nodules from the abdominal wall showed a dense perivascular infiltration throughout the dermis that consisted of mononuclear cells. Two histological types were seen. In one (Type A) there were many large lymphoid cells and atypical histiocytes. These atypical cells were large and possessed prominent hyperchromatic nuclei and some were multinucleated cells resembling the Reed-Sternberg cells (Fig. 2a). In the other (Type B) there were smaller atypical cells in the dermis which had cerebriform nuclei (Fig. 2b). In both types of lesions, parakeratosis and slight acanthosis was found. Electron microscopy of Type B lesions

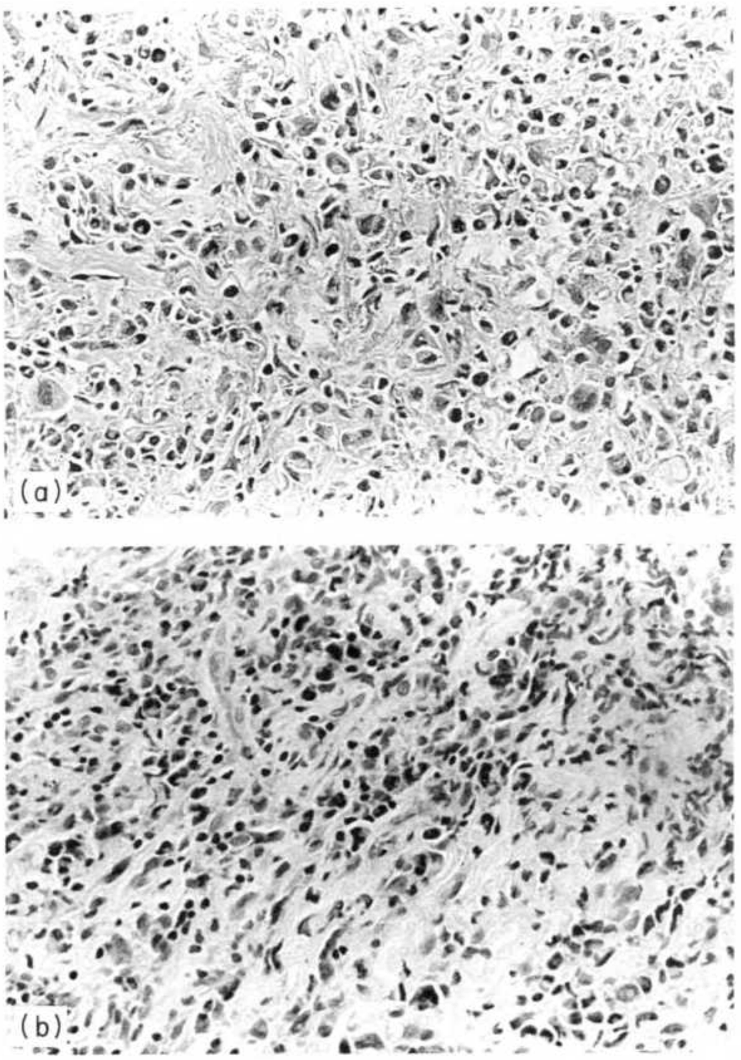

FIGURE 2. (a) Light microscopy of a necrotic nodule showing large lymphoid and atypical cells with prominent hyperchromatic nuclei (H&E stain, × 195). (b) Smaller cells with cerebriform nuclei in the dermal infiltrate (H&E stain, × 195).

showed that many of the mononuclear cells in the dermis had irregularly convoluted nuclei (Fig. 3), some with a cerebriform appearance.

The patient was treated once a week with PUVA therapy using topical applications of 0·3% 8-methoxypsoralen solution and subsequent irradiation of UVA. Despite this treatment, new papules continued to appear, but they did not develop into ulcerated and necrotic lesions.

METHODS

Skin samples

Five millimetre skin biopsy specimens were obtained from six of the papulonodular lesions well away from each other and processed for immunohistology. Three of six of the specimens were

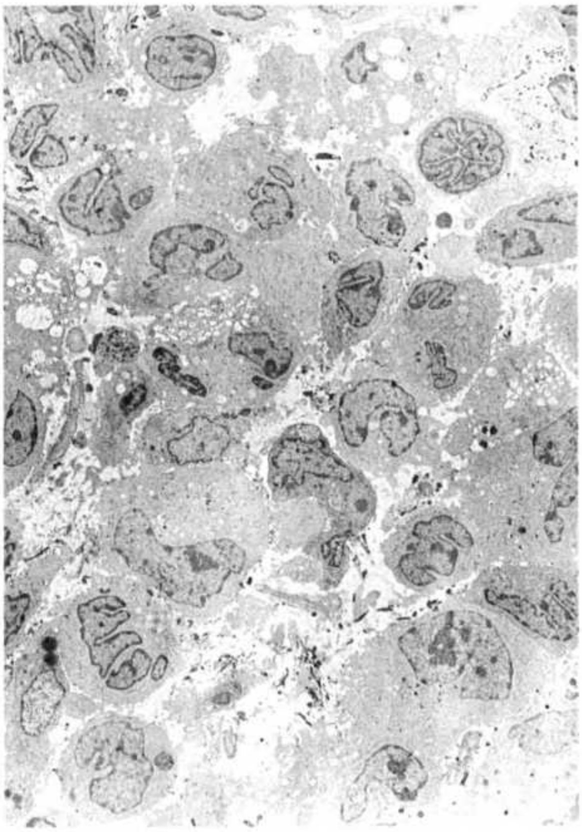

FIGURE 3. Electron micrograph showing lymphoid cells with convoluted nuclei in the dermis (× 1750).

divided immediately following their removal and one-half of each specimen was used for DNA hybridization studies.

Surface phenotypes

The surface antigens of the infiltrating cells were detected using an indirect immunoperoxidase procedure and the avidin–biotin–peroxidase complex method. Tissue specimens were fixed in periodic acid–lysine–paraformaldehyde solution, frozen and 6 μm sections cut which were then incubated with the following monoclonal antibodies: Leu 1, Leu 3a, Leu 8, anti-HLA-DR (Becton Dickinson FACS System, Sunnyvale, CA, U.S.A.), OKT 6, OKT 8, OKT 10, OKT 11 (Ortho Pharmaceutical Corp, Rarinton, NJ, U.S.A.) B1 (Coulter Electronics, Bethesda, MD, U.S.A.), anti-Tac, which is an antibody to the IL-2 receptor and reacts with activated and functionally mature T cells (provided by Dr Uchiyama. Kyoto University) and Ki-1 antibodies (Dakopatts, Copenhagen).

DNA preparation

DNA was extracted from 2 type A lesions and 1 type B lesion using standard procedures.[6] From 5 to 10 μg of the high molecular weight DNA samples were digested with restriction enzymes (*Eco*RI, *Bam*HI and *Hind*III), applied to 0·8% agarose gels and electrophoresed. Separated DNA segments were transferred to a nitrocellulose filter.[7,8] Hybridization was carried out at

68°C in a solution of 10% dextran, 0·1% sodium dodecyl sulphatase (SDS), 6 × SSC (1 × SSC is 150 mM sodium chloride and 15 mM sodium citrate, pH 7·0), 0·01% polyvinylpyrolidone (PVP), 5 μg of salmon-sperm DNA per ml, and 60 ng of ^{32}P labelled DNA fragment probes (10 cpm/μg DNA) specific to the human T-cell β-chain receptor gene. A *Hind*III digested lambda phage DNA was used for a molecular weight marker. The filter was washed at 68°C in 0·1% SDS, 0·1 × SSC solution several times, and then exposed to the X-ray film at −70°C.

Gene probe

The human T-cell beta-chain receptor gene probe consisted of a 3·0 kbp *Hind*III/*Eco*RI fragment of human DNA, encoding the constant region of the T-cell beta-chain receptor 1 (Cβ 1) which strongly cross hybridizes with the highly homogeneous Cβ 2 region. Rearrangements of both Cβ 1 and Cβ 2 are detectable only by this probe, which was cloned by Honjo *et al.*[9]

RESULTS

Surface phenotypes

Results of immunohistochemical staining of skin biopsy specimens are shown in Table 1. In four skin biopsies of Type A lesions there were large atypical cells with two or more nuclei which stained well with the anti-Tac monoclonal antibody (Fig. 4) and with anti Ki-1 antibody (Fig. 5), but not with Leu 1 or Leu 3a. However, the small lymphoid cells were reactive with Leu 1 and Leu 3a antibodies.

In the 2 Type B lesions there were cells that were similar and which possessed convoluted nuclei that stained well with Leu 1 and Leu 3a antibodies (Fig. 6), but did not react to anti-Tac antibody.

Clonality of infiltrating T cells

DNA hybridization studies did not reveal any rearrangement in the three specimens using *Eco*RI, *Bam*HI and *Hind*III. In non-rearranged germ line human DNA, two bands of 11 and 4·0 kbp in *Eco*RI fragments (Fig. 7), one band of 23·0 kbp in *Bam*HI (Fig. 8) and four bands of 12, 8, 6, 5 and 3·5 kbp in *Hind*III fragments (data not shown) were demonstrated.

TABLE 1. Immunohistochemical phenotypes of dermal infiltrating cells

	% of positive cells*							
Biopsy No. (Histological type)	OKT 11	Leu 3a	OKT 8	HLA-DR	OKT 10	anti-Tac	Ki-1	OKT 6
No. 1 (B)	70·5	64·3	19·9	73·7	0	0	0	8·6
No. 2 (B)	62·1	42·4	31·6	79·7	25·4	0	0	0·7
No. 3 (A)	51·0	43·4	21·7	42·5	41·2	6·3	1·5	2·2
No. 4 (A)	13·2	8·6	11·9	56·7	9·1	27·8	7·1	0
No. 5 (A)	25·0	20·6	13·5	48·3	32·2	30·3	6·9	0
No. 6 (A)	13·4	5·6	8·3	61·7	2·3	26·5	14·4	0·6

* Expressed as the percentages of positive cells to total mononuclear cells.

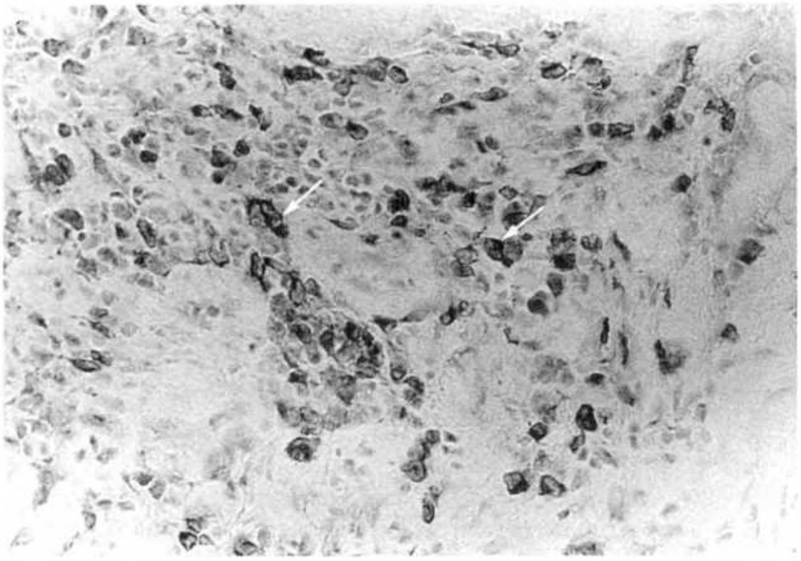

FIGURE 4. The immunohistology with anti-Tac monoclonal antibody showing large atypical cells that were well-stained with anti-Tac (arrows) (× 213).

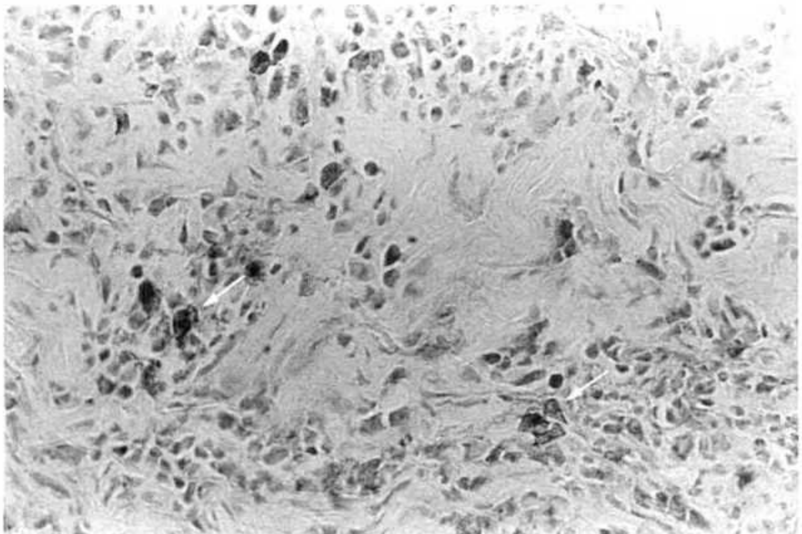

FIGURE 5. Section stained with anti Ki-1 antibody showing large atypical cells that were well-stained with this antibody (arrows) (× 213).

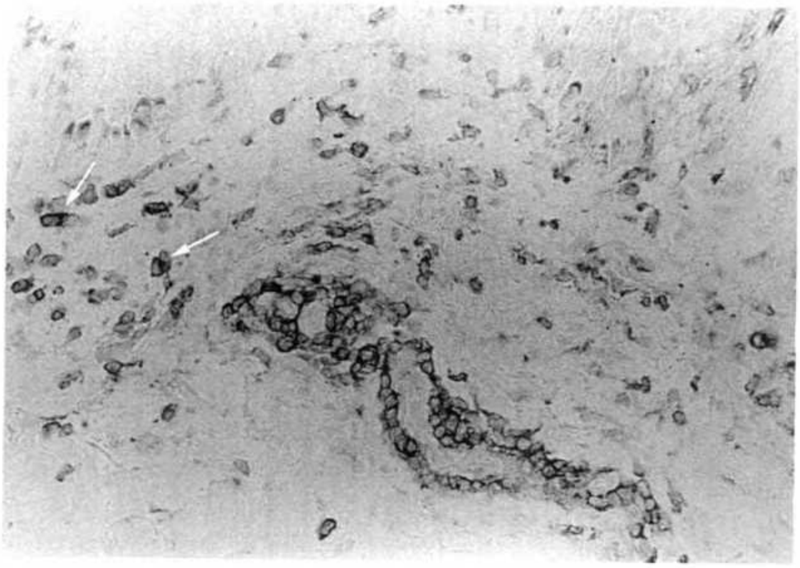

FIGURE 6. Section stained with Leu 3a antibodies showing medium and large sized cells with convoluted nuclei that were well-stained with Leu 3a (arrows) (× 213).

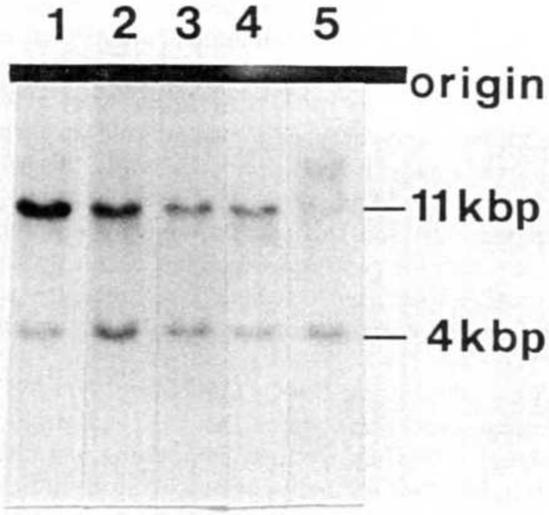

FIGURE 7. Autoradiograph of DNA analysis digested with *Eco*RI. Lane 1: negative control (normal peripheral blood mononuclear cells). Lane 2–4: three skin specimens from a single patient, Lane 5: positive control (peripheral blood mononuclear cells from an ATL patient).

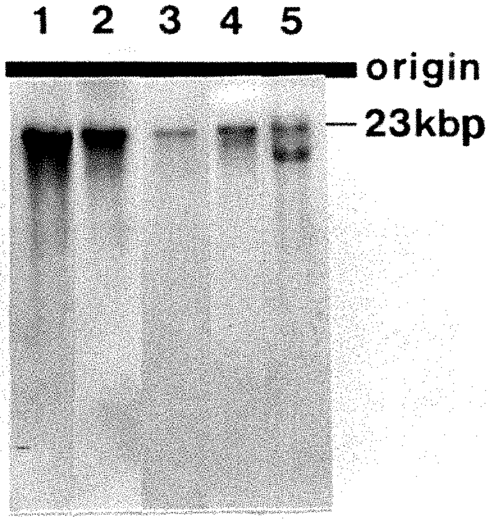

FIGURE 8. Autoradiograph of DNA analysis digested with *Bam*HI. Lane 1: negative control (normal peripheral blood mononuclear cells). Lane 2–4: three skin specimens from a single patient. Lane 5: positive control (peripheral blood mononuclear cells from an ATL patient).

DISCUSSION

Lymphomatoid papulosis has been described as having two different histological types, one in which there are large atypical cells resembling Reed–Sternberg cells (Type A) and another in which there are atypical cerebriform mononuclear cells as seen in the infiltrating cells in mycosis fungoides (Type B). It has been reported that both have different surface phenotypes.[10–12] It has been proposed that both types have the same origin despite the differences as observed on immunohistochemistry.[13] This is supported by staining of the atypical cells with Ki-1, which is a monoclonal antibody specific for Hodgkin's disease and Reed–Sternberg cells,[11–13] also the finding of transitional forms in some specimens with the histological features of both types.

We have demonstrated the two different types of histological features and surface phenotypes in the papulonodular lesions of a patient. As lymphoproliferative disorders have been found to develop in patients with lymphomatoid papulosis,[14,15] it is important to investigate the nature and origin of the infiltrating cells and this can be done by gene analysis, examining the rearrangement of the T-cell receptor gene and which has been used in the early diagnosis of malignant lymphoma.[16,17] Weiss reported that beta T-cell receptor gene rearrangement was found in five of six patients and three separate gamma T-cell receptor gene rearrangements were detected in one patient.[18]

Our DNA hybridization studies, using *Eco*RI, *Bam*HI and *Hind*III, have failed to show β T-cell receptor gene rearrangement. This suggests that the T-cell proliferations are non-clonal, unlike malignant lymphoma. However, a clone comprising less than 5% of the cellular infiltration may be undetectable. Further immunohistochemical studies and DNA analyses are needed on other cases to clarify the nature of lymphomatoid papulosis.

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Dermatoses in five related female carriers of X-linked chronic granulomatous disease

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SUMMARY

Eight female members of a family with X-linked chronic granulomatous disease were identified. Five were shown to be carriers of the disease gene. Each of these female carriers of the gene had a history of skin eruptions. The identification of the carrier state is important as genetic counselling should be offered and the prenatal diagnosis of this disorder is possible.

Chronic granulomatous disease (CGD) is a life-threatening immunodeficiency state. Affected individuals have a functional deficiency of the polymorphonuclear NADH oxidase system resulting in failure to kill phagocytosed catalase-positive bacteria and fungi. The condition is usually inherited as an X-linked trait and is frequently fatal in male infants. Normal neutrophils reduce nitroblue tetrazolium (NBT) whilst those from CGD patients fail to do so. Due to random X-chromosome inactivation (lyonization) female carriers of X-linked CGD have two populations of neutrophils; in one population the normal gene on the X-chromosome will be inactivated and these cells will therefore not reduce NBT, whilst in the other population the normal gene is expressed. Although females carrying this disease gene do not manifest a significant susceptibility to infection, dermatoses including photosensitivity, lesions resembling chronic discoid lupus erythematosus[1–4] and Jessner's lymphocytic infiltration[5] have been reported as well as severe aphthous ulceration.[1]

CASE REPORTS

The family was identified following the diagnosis of chronic granulomatous disease in a male infant (arrow, Fig. 1) who subsequently died with multiple hepatic abscesses. In order to determine the carrier status of female relatives they were investigated using a modification of the

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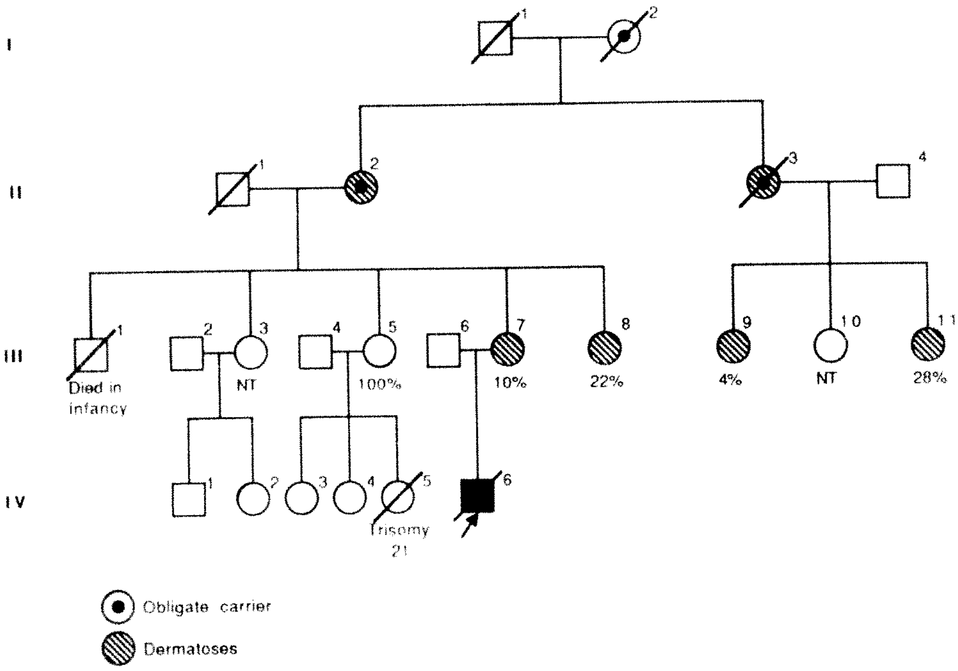


⊙ Obligate carrier

▨ Dermatoses

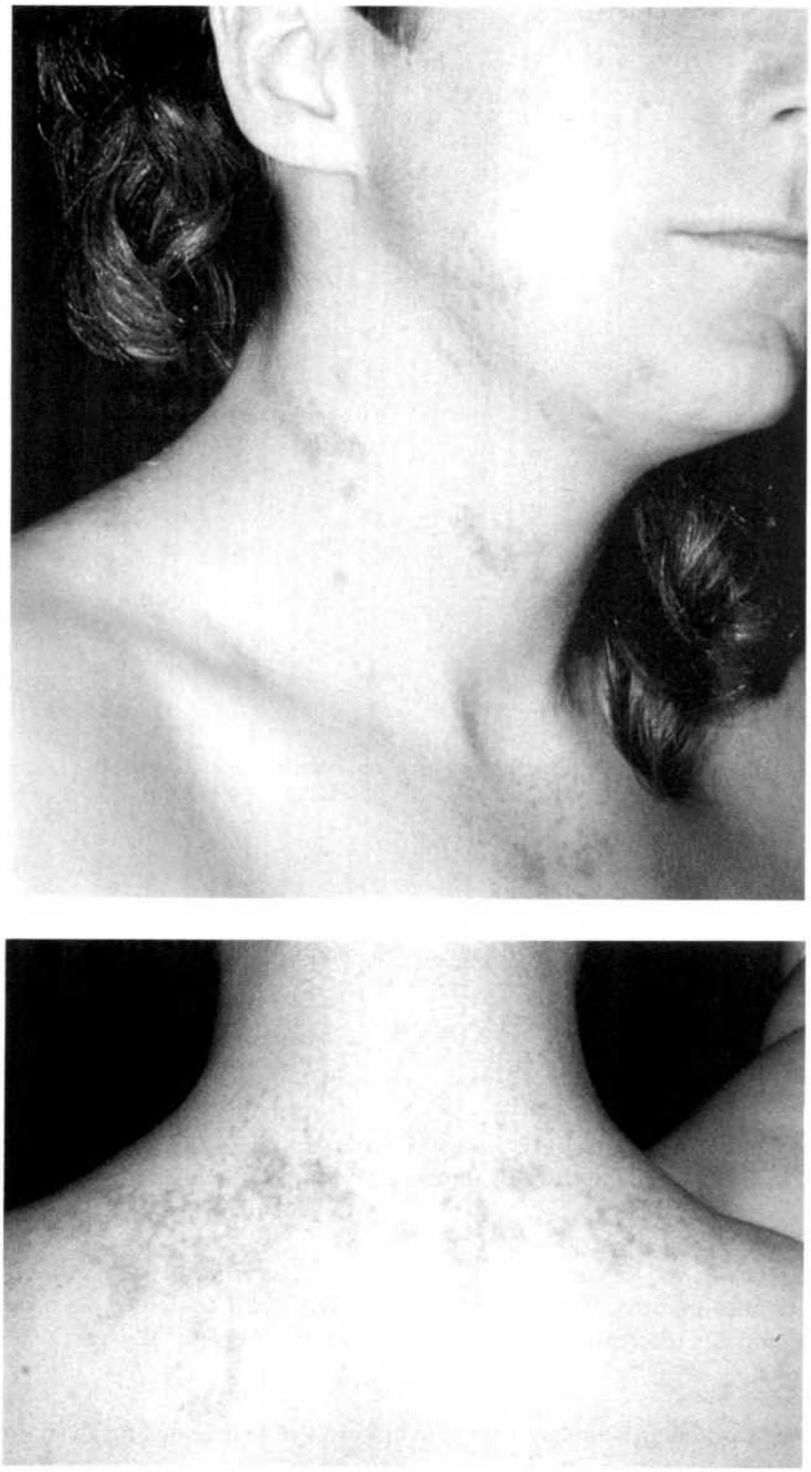
Subscripts (%) indicate proportion of Phorbol Myristate Acetate stimulated neutrophils reducing NBT. NT= not tested. Normal range 98-100%

FIGURE 1. Pedigree of family investigated.

NBT test as described by Levinsky *et al.*[6] Five females were shown to be carriers of this X-linked gene (Fig. 1).

Case 1 (Fig. 1, III.9)
A 27-year-old woman presented with persistent erythematous, raised and slightly scaly plaques that resembled chronic discoid lupus erythematosus on her face, neck and upper trunk (Figs 2 and 3). The lesions first appeared at the age of 2 and were worse during the summer months. Until her early twenties during the summer months she had a papular and erythematous rash with urticarial lesions and occasional blisters on the light exposed areas of her skin. These lesions took several days to clear. She had severe aphthous ulceration since early childhood. Routine haematology, biochemistry including porphyrins, serum immunoglobulins and complement (C4, FacB, C3c, C3d) were normal. Tests for rheumatoid factor, ANF, dsDNA, anti-Ro and anti-La antibodies were negative. A biopsy from one of the discoid lupus-like lesions on her upper back showed marked oedema of the upper dermis with a focal lymphoid infiltrate around dermal blood-vessels and adnexal structures. There were areas of basal cell liquefaction degeneration (Fig. 4). Direct immunofluorescence of this biopsy was negative.

Case 2 (Fig. 1, III.7)
A 33-year-old woman first developed erythematous patches below her eyes at the age of 1. They were more prominent in the summer months and persisted until her mid-teens. Recently she had been troubled with a pruritic, erythematous, maculopapular rash that occurred following sun exposure and took several days to clear. She suffered recurrent severe aphthous ulceration.



FIGURES 2 AND 3. Skin lesions on lower face, neck and upper back of case 1.

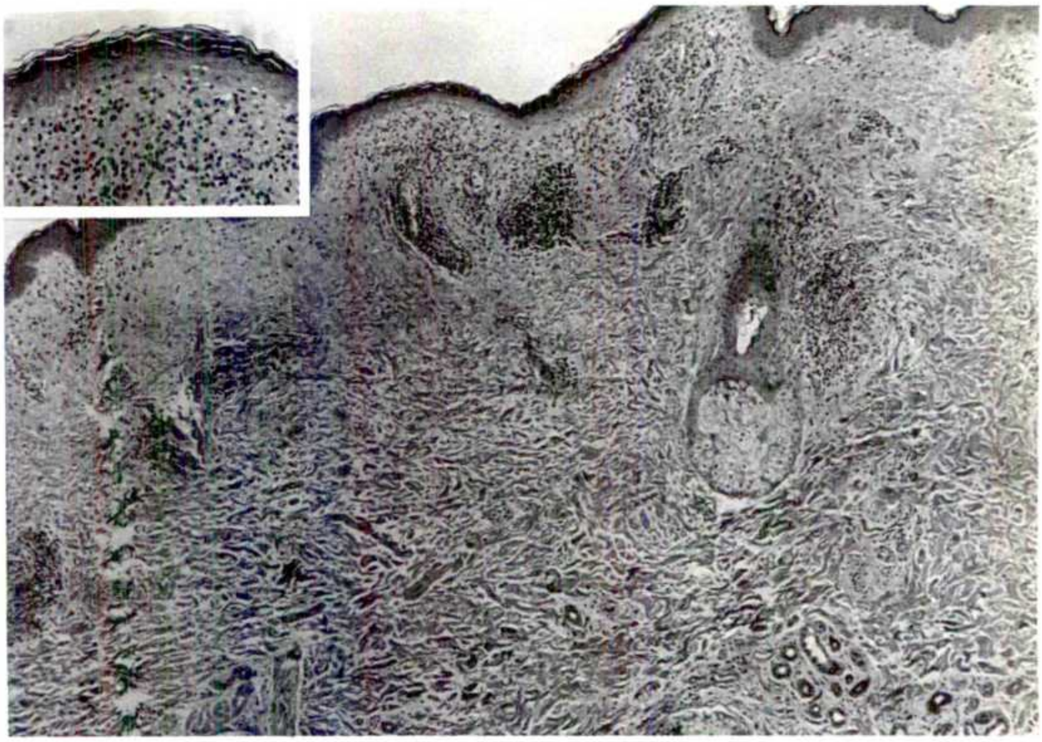


FIGURE 4. Skin biopsy from case 1. Lymphocytic infiltrate around dermal blood-vessels and hair follicles. There is focal epidermal atrophy and liquefaction degeneration of the basal layer (inset). H & E × 50 (inset × 100).

Case 3 (Fig. 1, III.8)
This 29-year-old sister of case 2 had similar problems with photosensitivity and aphthous ulceration when she was young. Following exposure to strong sunlight she developed erythematous maculopapular eruptions.

Case 4 (Fig. 1, III.11)
The 24-year-old sister of case 1 had, as a child, similar though less severe skin lesions. As she became older they occurred only during the summer months and had resolved completely by her mid-teens. Like her sister, she had severe and recurrent aphthous ulcers when younger.

Case 5 (Fig. 1, II.2)
At the age of 24 years this woman started to develop raised, erythematous plaques below her eyes during the summer months. This occurred for 20 years and coincided with her residing in southern England. On returning to Scotland with its poorer sunshine she was no longer troubled by these lesions.

The routine blood investigations carried out on case 1 were repeated for the other cases, these also showed normal results.

Three female members of the family denied ever having had skin problems or mouth ulceration. One (Fig. 1, III.10), a sister of case 1, now lives in Israel and was not investigated. The second (Fig. 1, III.5) had a single population of normal neutrophils. The third (Fig. 1, III.3) has declined to be investigated.

DISCUSSION

We have identified five related carriers of X-linked chronic granulomatous disease (CGD). Each carrier gave a history of skin disorder though the severity and duration varied considerably. The most severely affected had persistent lesions resembling chronic discoid lupus erythematosus since early childhood and up until her early twenties suffered polymorphic light eruption and severe aphthous ulceration. The more mildly affected members of the family experienced photosensitivity and aphthous ulceration until their mid-teens.

Why female carriers of X-linked CGD should develop a spectrum of dermatological manifestations is not known. It is unlikely to be due only to a deficit of functional neutrophils as affected males do not show the same propensity for skin changes. Subcutaneous abscesses and hidradenitis suppurativa have been reported in cases of X-linked CGD and it has been proposed that a resulting chronic antigenaemia might eventually provoke autoantibody formation and thus, the observed skin changes.[4] However, none of the carriers in the family reported here gave a history of such infections and furthermore autoantibodies were negative in all cases. There did, however, appear to be a correlation between the proportion of defective neutrophils and the severity of the skin changes (Table 1) which may therefore reflect the degree of lyonization. In the NBT test used to identify the carriers in this family the neutrophils were stimulated by phorbol myristate acetate. This form of NBT test is believed to be a more reliable form of identifying carriers of the chronic granulomatous gene.[6] Occasionally CGD is inherited as an autosomal recessive trait and this form may present later in life. Facial lesions resembling chronic discoid lupus erythematosus have been reported in a 23-year-old male whose neutrophils showed a defective oxidative burst. Both he and his sister, who had CGD, were reported as having lupus-like lesions on their fingers.[7]

In two previous studies a total of 53 female patients with discoid lupus erythematosus were assessed for carriage of CGD and no carriers were found.[8,9] We suggest that the carrier status for this disorder should be checked in females with CDLE-like lesions when there is a family history of male infant death, frequent and recurrent infections or when several female members in a family suffer from dermatoses as in this family. Female carriers should be offered genetic counselling and prenatal diagnosis is possible in at-risk male pregnancies.[10]

TABLE 1. Correlation of NBT scores and severity of symptoms

Case	% of phorbol myristate acetate stimulated neutrophils reducing NBT	
Case 1 (Fig. 1, III.9)	4%	Increasing severity and duration of symptoms
Case 2 (Fig. 1, III.7)	10%	
Case 3 (Fig. 1, III.8)	22%	
Case 4 (Fig. 1, III.11)	28%	

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Blistering, erosions and scarring in a patient on etretinate

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SUMMARY

A case is reported of a patient who developed blistering of the skin followed by ulceration and scarring while on treatment with etretinate.

Skin fragility with etretinate is well described.[1,2] We report a patient who developed blistering and erosions whilst on this drug.

CASE REPORT

A 63-year-old lady who had life-long chronic plaque psoriasis and was poorly tolerant of anthralin was started in September 1986 on etretinate, 1 mg/kg per day. Her rash cleared and remained so enabling the dose to be reduced to 0·5 mg/kg per day in December 1986. She later developed discrete areas of spontaneous ulceration and bleeding of the skin on her abdomen and right leg. These healed initially with potassium permanganate soaks and magenta paint, but over the next few weeks broke down again and similar lesions appeared on the skin of the lower back, buttocks and left leg. In August 1987 the area on the right thigh showed haemorrhagic blisters. The erosions increased in size despite continuing topical treatment and in January 1988 she was admitted to hospital. She was clear of her psoriasis and her only other complaint was that of brittle nails. She was then taking etretinate 0·5 mg/kg per day having been on the drug for a total of 16 months. On examination, there was over her right lateral upper thigh a 5 × 7 cm superficial, angular shaped ulcer with numerous smaller erosions over her abdomen and inframammary area. (Fig. 1). Hypertrophic granulation tissue was present within many of the erosions. On the skin over the lumbar spine and abdomen there were numerous small oval and linear white scars at the sites of the earliest lesions. There was no evidence of nail dystrophy or hypergranulation round the nails and no buccal mucosal ulceration. The etretinate was stopped on admission and

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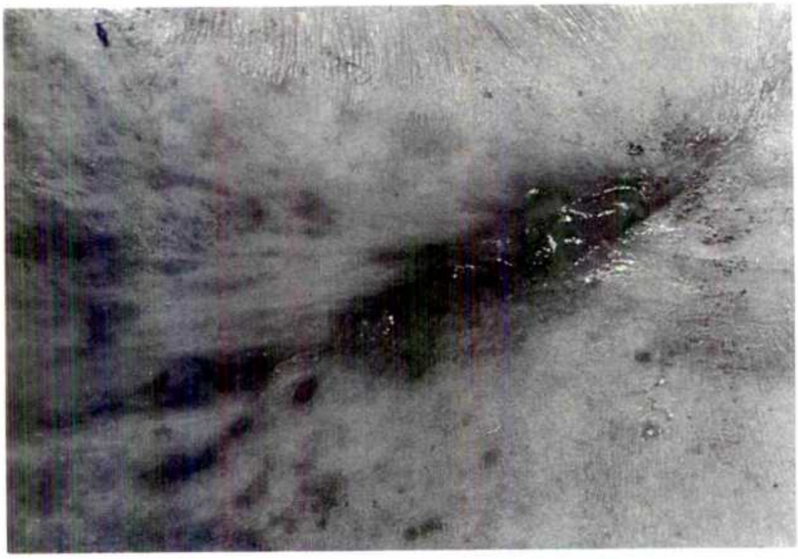

FIGURE 1. Erosions over inframammary area.

10 days later the erosions were improving and 36 days after stopping etretinate all had healed. Her psoriasis returned and has slowly increased in extent.

Histology

Biopsies were taken on three separate occasions from eroded skin on the right thigh, buttock and iliac crest. No intact blister was seen. Microscopically there was parakeratosis, spongiosis and incipient clefting at the dermo-epidermal junction (Fig. 2). Within the dermis, there was an infiltration of plasma cells, eosinophils and neutrophils. The dermal tissue was scarred in areas adjacent to this infiltrate. Another feature was the presence of granulation tissue at the sites of ulceration and the accumulation of fibrinoid material within the dermis. Stains for mucin were negative.

Suction blister time

Because of some resemblance of the clinical findings to the blistering of the skin of patients treated with PUVA the suction blister time was measured in our patient 1 week after stopping the etretinate. The method was as previously described[3] and the time to complete blistering was 28 min which is markedly reduced when compared with that of control subjects (mean 76·7 ± 3·0 SEM) and even shorter than in PUVA induced blistering (mean 42·5 min).

Plasma etretinate level

This was measured 10 days after cessation of treatment and 1 month later when the results were 145·4 mg/ml and 100·8 mg/ml respectively. Although there is considerable patient–patient variation in etretinate levels, the initial one of 145·4 mg/ml is higher than the expected range of 50–100 mg/ml (G. Fothergill, personal communication).

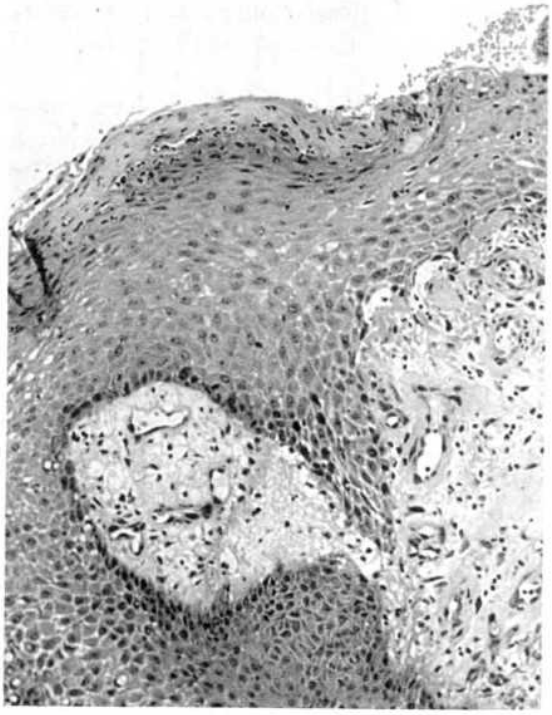

FIGURE 2. Dermo–epidermal clefting and epidermal spongiosis.

Other investigations

Liver function tests, porphyrin screen, VDRL urinary hydroxyproline and virological and bacteriological examination of erosions were normal or negative.

DISCUSSION

The excess granulation tissue and the nail brittleness exhibited by out patient are well known side-effects of etretinate. Skin fragility is also well described though ulceration and blistering have rarely been mentioned. Blistering of the skin has been reported previously twice and ulceration only once.[1,2] The scarring seen on our patient is, as far as we know, unique. It is not possible to be sure of the localisation within the skin of the primary damage. Reduction in suction blister time is classically associated with separation at the dermo–epidermal junction though it need not necessarily be the primary event. The situation is similar to that in PUVA induced blisters where the suction blister time is reduced and subepidermal separation and epidemal necrosis are described. Experimental work in mice has suggested the primary effect of retinoids is on the epidermis and this remains a possible explanation in our patient.[4] The scarring points to additional dermal damage and its relationship to and the nature of the fibrinoid material found in the dermis remains obscure. It is known that retinoids affect mesenchymal tissues including cartilage and connective tissue.[5,6]

The side-effects of etretinate on the skin are generally related to dosage and the first plasma level in our patient (145·4 mg/ml) was higher than expected (50–100 mg/ml). The fall to 100·8 mg/ml a month later when the skin had improved would suggest there is a threshold below which the clinical side-effects are not apparent and the fact that the etretinate is still present in some quantity a month after stopping the drug is in keeping with its long half-life and its deposition in adipose tissue.[7]

A previously reported patient with etretinate-induced blisters[1] had cirrhosis of the liver and slow elimination of the drug was thought to contribute to its toxicity, though plasma levels were not measured until the patient had been off the drug for 2 months. Our patient's liver function tests were normal and in the absence of more data on other subjects we cannot say whether the plasma levels were inordinately high or the skin was in some way particularly susceptible to levels commonly recorded in patients on etretinate.

ACKNOWLEDGMENT

We are grateful to Dr A.J.Shipman, Head of Medical Department, Roche Products Ltd., for measuring the etretinate levels.

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Exfoliative dermatitis due to nifedipine

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SUMMARY

We report the case of a 63-year-old Caucasian female, with reno-vascular hypertension, who developed a generalized skin eruption with lichenoid histological features, induced by nifedipine. A repeat challenge with nifedipine confirmed that it was the causative agent.

Nifedipine is a calcium channel blocker, which relaxes arterial smooth muscle and is commonly used in the treatment of angina, hypertension and Raynaud's phenomenon. This drug is normally well tolerated, although minor side-effects associated with peripheral vasodilation occur in approximately 20% of patients.[1] Gravitational oedema associated with increased capillary filtration has been reported.[2] Skin reactions are extremely rare and are not mentioned in the data sheet.

CASE REPORT

A 63-year-old woman attended her general practitioner in February 1988 with an exacerbation of her bronchitis. Her blood pressure (BP) was significantly raised at 260/140 mmHg and therapy with nifedipine sustained release (SR) 10 mg b.d. was initiated. Spironolactone 100 mg b.d. was added 2 weeks later, with atenolol 50 mg daily after a further 8 days, as her BP remained high.

Later in April her BP was 210/95 mmHg and a widespread macular rash was noted. Nifedipine and spironolactone were discontinued and captopril 12·5 mg b.d. was started. The rash improved initially but a week later she was admitted with severe erythroderma, malaise, shivering and polydipsia.

There was a past history of hypertension during her only pregnancy 30 years previously, and of peripheral vascular disease with intermittent claudication of both calves on walking 100 m. Her family history was non-contributory. She smoked 10 cigarettes daily.

On examination she had an exfoliative dermatitis of the trunk, limbs and palms with relative sparing of the face. The groins and flexures were weeping and crusted. There was no mucous membrane involvement. She was pyrexial at 38·5°C and her BP was 260/120 mmHg.

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Fundoscopy was normal. There were no signs of cardiac failure. Peripheral pulses below the femoral arteries were absent.

Investigation

Plasma creatinine, urea and electrolytes were normal apart from a low plasma potassium of 2·6 mmol/l. Plasma viscosity was raised at 1·82 cp (normal 1·5–1·72). Serum alkaline phosphatase was raised at 15 KA units (normal 3–13) with a slight increase of the hepatic free fraction on isoenzyme analysis; liver function tests were otherwise normal. Autoimmune profile was negative apart from weakly positive antibodies to gastric parietal cells, intrinsic factor antibodies being negative.

An intravenous urogram showed small kidneys consistent with generalized vascular disease. Skin swabs yielded a heavy growth of *Staphylococcus aureus*, β-haemolytic *Streptococcus* group B and Gram negative bacilli. The following investigations were normal or negative: full blood count, complement studies, urinary porphyrins, resting and standing plasma aldosterone and renin levels, plasma pentolinium test, chest X-ray, abdominal computerized tomography and meta-iodobenzylguanidine (MIBG) scan. The evidence pointed to a reno-vascular cause for her hypertension and a phaeochromocytoma and Conn's syndrome were excluded.

Histology

A skin biopsy taken from the forearm showed mild hyperkeratosis with areas of parakeratosis and some spongiosis of the lower epidermis. Necrotic epidermal cells with eosinophilic cytoplasm and pyknotic nuclei were prominent, particularly in the basal layers. Eosinophilic colloid bodies were also present within the epidermis. Focal areas of basal layer liquefaction were seen. The upper dermis showed mild oedema and contained a mononuclear cell infiltrate which although being predominately perivascular (Fig. 1), was also band-like in some areas. Direct immunofluorescence was negative. These appearances were considered to be consistent with a lichenoid drug reaction.

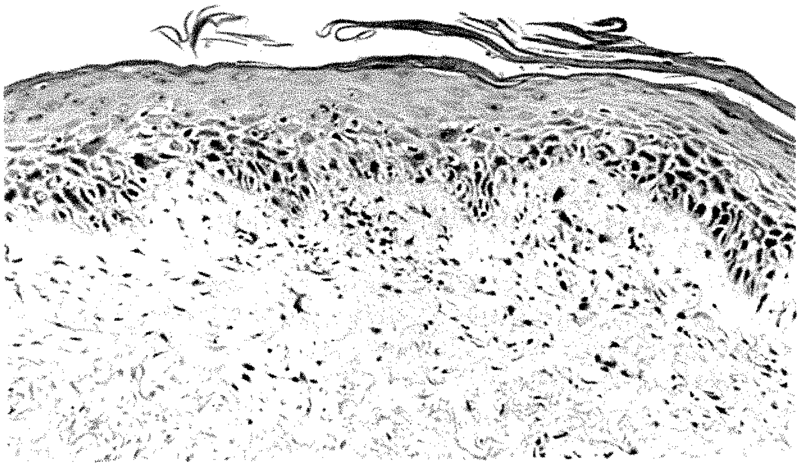

FIGURE 1. Skin biopsy showing parakeratosis, epidermal cell necrosis, colloid bodies, focal basal layer liquefaction and a predominately perivascular mononuclear infiltrate.

Progress

Atenolol was stopped on admission, this being the only drug which remained from the time when the rash first appeared. Captopril was continued and Dyazide® (triamterene 50 mg, hydrochlorothiazide 25 mg) one tablet daily was introduced. Treatment for her skin condition consisted of dilute topical steroids, emollients and erythromycin 500 mg q.d.s.

Her skin improved but her BP remained poorly controlled and so nifedipine SR 20 mg b.d. was reintroduced on her eighth day in hospital, with good effect. Interpretation of the timing of events, i.e. deterioration of our patient's rash after the nifedipine had been stopped and the lack of previous reports of adverse skin reactions to nifedipine, were important influences in this decision. Her condition stabilized to allow discharge home on her thirteenth day in hospital. Ten days later however, she was readmitted with an exfoliative erythroderma associated with diffuse alopecia. Methyldopa 250 mg t.d.s. was substituted for nifedipine and within 2 weeks her rash had completely settled. Her unstable BP came under control and all other antihypertensive drugs were subsequently discontinued. She inadvertently received a further single dose of 20 mg nifedipine SR provoking a recurrence of the rash 24 hours after which faded within a week. A further skin biopsy after this rechallenge showed similar, though less marked lichenoid changes.

DISCUSSION

Our patient developed a widespread exfoliative dermatitis 2 months after the commencement of nifedipine, spironolactone and atenolol which recurred when nifedipine was reintroduced. To our knowledge such a severe reaction to nifedipine has not been previously documented.

Reports of localized cutaneous reactions to nifedipine include erythematous oedema of the legs,[3] erythromelalgia,[4,5] leucocytoclastic vasculitis[6] and photosensitivity.[7] Generalized skin eruptions caused by nifedipine are not well documented in the literature, there being to our knowledge only two previously reported cases,[8,9] where rechallenge allowed confirmation of the diagnosis. Alcalay *et al.*[8] described a generalized bullous eruption in an 80-year-old man occurring several months after starting nifedipine. Histology showed eosinophilic necrosis in the epidermis and a moderate perivascular mononuclear infiltrate in the dermis. A strong linear deposition of fibrin at the dermo–epidermal junction was present on direct immunofluorescence. Recently a patient has been described who developed erysipelas-like lesions on both shins with several small scaly erythematous plaques over the trunk shortly after the introduction of nifedipine.[9] The histological features of epidermal hyperplasia, spongiosis, exocytosis and an upper dermal infiltrate were considered to be suggestive of a lichen planus-like drug eruption. An additional case was reported[10] where the time course of events suggested an association between the introduction of nifedipine and the development of an exfoliative dermatitis, but rechallenge was not undertaken. Furthermore, Findlay[11] described six elderly women who developed a distinctive purpuric morbilliform eruption, four of whom were taking nifedipine; withdrawal or readministration of this drug, however, made no apparent difference to the course of the illness.

Although our patient presented with the clinical picture of an exfoliative dermatitis the histological features were similar to those previously described in lichenoid drug eruptions,[12,13] lichenoid reactions being classically defined by their characteristic histology despite variation in the overall clinical appearance.[14] We would suggest, therefore, that nifedipine should be added to the list of drugs[15] known to provoke lichenoid reactions.

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Prolidase deficiency: a case report and literature review

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SUMMARY

We describe a patient in whom chronic leg ulceration was due to prolidase deficiency. The clinical features of this condition are described and we discuss the metabolic abnormality and the treatment regimes which have been employed. We also report the further finding of erosive cystitis, which we consider should be added to the list of clinical features of prolidase deficiency.

Prolidase deficiency is a rare, recently described, inherited metabolic disorder. Substantial loss of dipeptides containing proline and hydroxyproline occurs in urine (iminodipeptiduria) and tissue prolidase levels, as assayed in erythrocytes, leucocytes and fibroblasts, are low.[1] Affected individuals display a number of characteristic features, especially skin lesions, although prolidase deficiency can occur with no clinical manifestations.

CASE REPORT

A female born in 1942 first presented to dermatology out-patients in 1960 with a 6-month history of bilateral leg ulcers. Apart from treatment for pulmonary tuberculosis at the age of 10 years there was no other relevant personal or family history. Examination revealed oedema of both legs with extensive ulceration (Fig. 1) and areas of atrophie blanche and multiple telangiectases on lower legs and thighs. She was noted to have an odd facial appearance, described then as 'bird-like', with epicanthic folds, an unusual body habitus with narrow shoulders and broad hips, spongy skin texture and elbows which could hyperextend by 10°. Levels of proline and hydroxyproline in urine were elevated. Chromosome studies were normal. A provisional diagnosis of a collagen disorder, possibly Ehlers–Danlos syndrome, was made.

Response to out-patient treatment was poor, and she was therefore admitted to hospital. To exclude artefactual damage the limbs were occluded for 3 weeks but the ulcers increased in size. Only after a lengthy admission with prolonged bed rest did improvement take place but deterioration occurred following discharge and this was the pattern in successive years.

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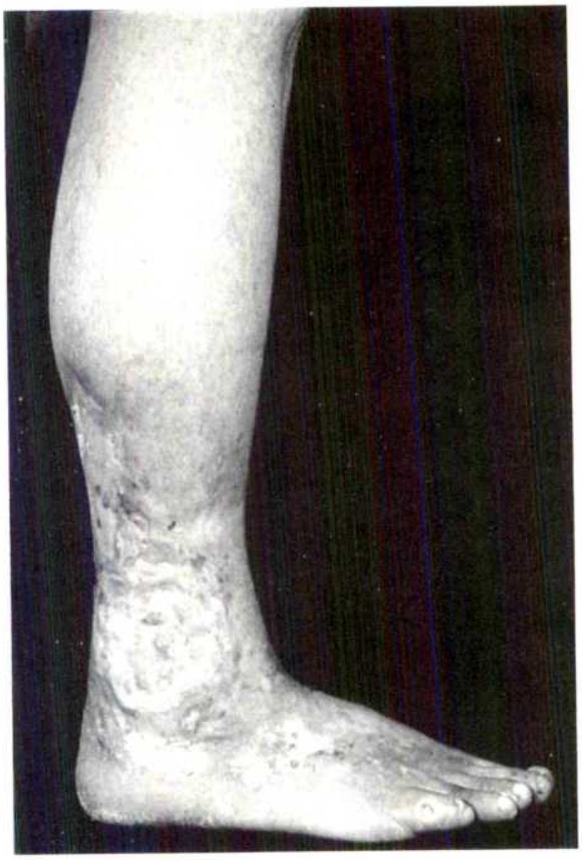

FIGURE 1. Ulceration of the right leg.

Further investigations carried out during subsequent admissions in several different centres included venography, which we understand showed paucity of valves in long and short saphenous veins and large communicating veins, and lymphangiography which showed no abnormality. A mild hypochromic, microcytic anaemia was found on several occasions.

Skin biopsies showed non-specific inflammatory changes. However, a histology report in 1971 suggested that dermal collagen bundles were thickened and collagen studies showed that both affected and unaffected skin had a very low collagen content. Electron microscopy showed the unaffected skin to have a normal appearance but affected skin collagen had a very marked cross banding pattern and was described as looking 'shredded'. These findings were not, however, considered diagnostic.

An additional problem since 1974 has been recurrent episodes of urinary frequency and urgency, with right loin pain. Urinalysis has revealed haematuria, proteinuria and pyuria but no organisms. In view of her past history she was investigated for renal tract tuberculosis but no mycobacteria were found after careful and repeated searches. Cystoscopy showed gross generalized cystitis. A biopsy showed chronic inflammatory changes only. There have been further episodes to the present day with organisms found in the urine only on infrequent occasions.

In 1981, following reports in the literature of a newly described abnormality, prolidase deficiency, urine dipeptides were assayed and shown to be massively increased. Red cell prolidase was assayed and found to be very low (< 7% of normal). The fibroblast prolidase level was also low, confirming prolidase deficiency.

The immediate members of our patient's family, her mother, two brothers and a sister, were screened for evidence of the condition. All were clinically normal. One brother had a red cell prolidase level of the same order as our patient and an elevated IgG level. The second brother had a reduced level of red cell prolidase of approximately 50% of normal. Her sister and mother had normal levels. Her father had died some years before and there was therefore no data on him. Our patient's biochemical data have been reported previously.[2]

DISCUSSION

In 1968 Goodman[3] described a new condition in a male patient with mental subnormality, physical abnormalities and leg ulceration in association with iminodipeptiduria. It was later suggested that this was due to prolidase deficiency.[4] Powell confirmed prolidase deficiency in a patient of his by enzyme assay in 1974—the first time this had been performed.[5] To date, 21 cases have been reported including our patient and her brother, although in only 19 have prolidase estimations been performed.

The enzyme prolidase is widely distributed in the tissues of man.[1] It acts by cleaving dipeptides with a C-terminal proline or hydroxyproline. Proline is recycled and used in further protein synthesis, hydroxyproline is excreted in urine (Fig. 2). The complementary enzyme prolinase acts on dipeptides with an N-terminal proline/hydroxyproline. Prolinase levels and activity remain normal in prolidase deficiency and indeed no abnormality of prolinase has ever been described. By these pathways 90–95% of proline is conserved.[6] Absence of prolidase therefore depletes the total pool of proline which is lost in large amounts in urine bound to amino acids as iminodipeptides, e.g. glycyl–proline. Although iminodipeptiduria has been described in other conditions, e.g. hypophosphataemic (Vitamin D resistant) rickets[7] and hyperparathyroidism,[8] due in both cases to increased collagen breakdown, it only occurs to such a massive degree in prolidase deficiency.

Proline and hydroxyproline together constitute 20–25% of collagen.[1,9] In addition proline is

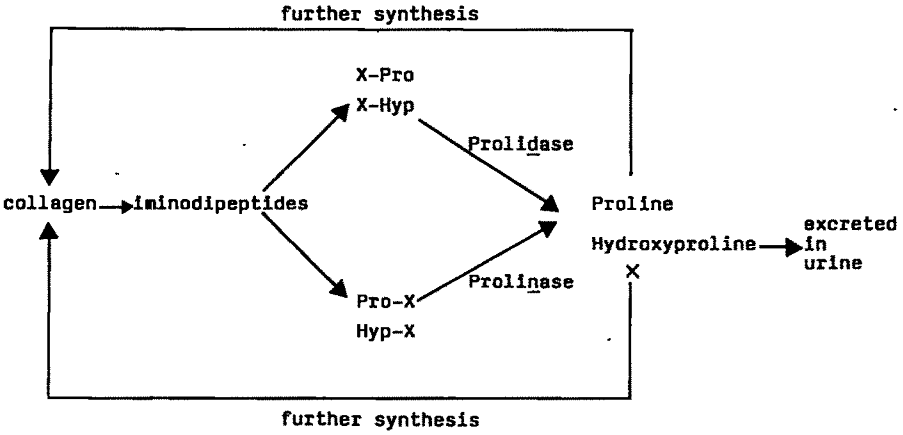

FIGURE 2. Pathway of collagen metabolism showing action of prolidase. X = amino acid.

TABLE 1. Clinical features of patients with prolidase deficiency

Skin	Leg ulceration Scarring and pitting } frequently involving Telangiectases or purpura } thigh as well as lower leg Lymphoedema (due to chronic inflammation?) Premature greying of hair
Central nervous system	Low IQ
Musculo-skeletal	Lax abdominal wall and protrusion of abdomen Hyperextensible joints
Other physical characteristics	'Characteristic facies' Hypertelorism Depressed nasal bridge Splenomegaly Recurrent infection especially otitis media and upper respiratory tract infection

found in other peptides, e.g. thyrotropin releasing hormone (TRH)[1] and complement fraction C1q.[10] Loss of proline in prolidase deficiency has been estimated at as much as 3 g daily.[9] There has been no estimate of the daily loss of other amino acids in prolidase deficiency. Prolidase can be assayed in erythrocytes, white cells and fibroblasts, and levels are very low or undetectable in affected individuals. Activity can also be measured in cultured amniotic cells so that pre-natal diagnosis may eventually be possible.[1]

A number of clinical features have now been reported and these are shown in Table 1. These are not constant but are the most common abnormalities found in cases so far described.

The most consistent features are skin fragility with leg ulceration and characteristic pitting and scarring. These were present in our patient. Histology of affected skin shows non-specific changes. Changes seen on electron microscopy also tend to be non-specific but Leoni found that the mean diameters of collagen fibres in two affected individuals were smaller than controls.[11]

In addition, other features have been noted in a few individuals including hyperkeratotic skin lesions, pruritus, photosensitivity, arched palate, simian crease, wasting of small muscles of the hand, short stature, talipes equinus, osteoporosis, deafness and ocular keratitis. The most consistently abnormal laboratory findings are mild anaemia (hypochromic and microcytic), thrombocytopaenia and hypergammaglobulinaemia.

Although our patient's family history is not entirely consistent with this, other reports suggest the condition is inherited as an autosomal recessive trait.[12] Heterozygotes show no clinical abnormality and do not display iminodipeptiduria but prolidase levels are reduced to approximately 50% that of controls. However, it is important to note those cases reported of individuals with biochemically established prolidase deficiency with no clinical features of the disease,[13,14] among whose number is our patient's brother, thus illustrating the variability of expression of the defect.

An additional feature of our patient's condition is recurrent cystitis with proven inflammatory changes seen on cystoscopy and confirmed histologically. Although recurrent infection is a feature of prolidase deficiency this usually involves the upper respiratory tract. Urinary tract infection has not been documented as a feature. In any case infection as a cause for our patient's symptoms has not been established. We believe that erosive cystitis may be a direct consequence of the disease and should be added to the list of possible clinical features.

Treatment methods usually concentrate on the skin lesions as these are often the most troublesome feature of the disease. Standard treatment regimes employing bed rest, occlusive dressings, topical antiseptics and skin grafting have a very limited and temporary effect. Our patient was treated on two occasions with intravenous dextran and her ulcers showed a favourable response. However, following an allergic reaction to the third course of treatment, no more was given.

A variety of measures aimed at compensating for the metabolic abnormality has been tried. Blood transfusion to correct anaemia also increases prolidase in red cells. This effect has been shown to last several weeks but produces only a temporary effect on the skin.[11] Proline has been tried both as a dietary supplement and as a topical preparation but with no apparent improvement.[12,13] However, one young patient who was treated with proline, manganese (which activates prolidase)[1] and Vitamin C (employed presumably for its effect on collagen synthesis) all orally, showed improvement, not only in the condition of her skin, but also in her height, bone age and IQ.[15] There was no observed change in proline excretion during the treatment period. A topical preparation containing glycine and proline has been used successfully to heal ulcers which had resisted all previous attempts.[16]

Patients who develop leg ulceration at an early age and who have a family history of leg ulcers should be examined for clinical features of prolidase deficiency and should have the appropriate investigations.

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Correspondence

Effects of alprazolam on platelet activating factor-induced wealing

SIR, Platelet activating factor (PAF) may be an important mediator of inflammation in the skin.[1] As PAF is labile and difficult to assay, a clinically safe and selective PAF antagonist could provide a useful tool in the further investigation of its role in skin disease. The triazolobenzodiazepine alprazolam has been shown to specifically block PAF induced platelet aggregation *in vitro*[2] and to inhibit anaphylaxis in guinea pigs.[3] We have now assessed the effect of alprazolam on weal and flare reactions to PAF and histamine in the skin of seven healthy non-atopic adults (age range 23–40).

Intradermal injections were performed in the volar forearm in randomized fashion using 25 gauge needles and 50 μl of phosphate-buffered saline as the vehicle. Injections were of 9 nmol (1 μg) histamine (Sigma St. Louis, MO, U.S.A.), 100 and 200 pmol C_{16} PAF (Bachem), and vehicle alone as control, and were performed 1 h before and 2 h after taking 1 mg alprazolam tablets (Upjohn Pharmaceuticals, Kalamazoo, MI, U.S.A.) by mouth. The area and thickness of the weals were measured by planimetry (Planix), sprung thickness gauge (Mitotoyo, Japan) respectively 10, 15, 20, 30 and 60 min after each injection and the results expressed as the area under the curve (AUC) when plotted against time after subtraction of the control value (Table 1).

TABLE 1.

AUC (mean ± SD)	PAF 100	PAF 200	Histamine
Area (cm/min)			
Before alprazolam	55 ± 16	73 ± 63	88 ± 65
After alprazolam	47 ± 15	42 ± 17	41 ± 9
Thickness (mm/min)			
Before alprazolam	33 ± 16	39 ± 21	54 ± 36
After alprazolam	29 ± 11	41 ± 17	42 ± 15

No significant differences in size or duration of wealing were found after alprazolam (Student's *t*-test). The expected plasma level of alprazolam following the therapeutic dose (1 mg) used (approximately 0·06 μmol from published values[4]) falls substantially below levels used in the *in vitro* study (2–40 μmol).[2] The dose we used caused significant drowsiness in all subjects and higher doses would not be tolerated by volunteers. In view of this limitation alprazolam may not be a useful PAF antagonist in man. However, recent reports of major qualitative differences in the response of atopic subjects to intradermal injections of PAF[5] suggest further studies of alprazolam would be of interest in this group of subjects.

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Recurrent hypothermia in a West Indian

Sir, The great majority of English language textbooks of dermatology are written by white dermatologists observing predominantly white patients. Patterns of response as seen in dark skins may be very different, and in particular the absence of erythema as a response to inflammation may allow severe skin disease to be overlooked. A case is described of a black patient with recurrent hypothermia in whom the diagnosis of an exfoliative dermatitis was only belatedly appreciated. Treatment of the skin disorder resulted in a return to normal thermoregulation.

In January 1987 a 65-year-old West Indian man presented to the Accident and Emergency department in a collapsed state, with a rectal temperature of 29°C. He recovered in hospital and was discharged. In April 1987 the patient was again admitted as an emergency case suffering from hypothermia, with a temperature of 31°C. The houseman's case notes at that time record him as an 'unkempt neglected type of person'. After 3 days on the ward his temperature was still only 34°C. Investigations included a normal brain scan, and blood cortisol level. His T4 level was low on several occasions, but the TSH was not elevated. His skin was noted by the physicians to be scaling; a past history of eczema was elicited from the case notes, and a variety of emollients and topical steroids were applied without apparent benefit. He remained on the ward, lethargic and disinterested, from April until August because of problems of placement. Throughout this time his temperature was never recorded as being above 35·5°C. He was discharged in August, but readmitted in September with hypothermia.

In November 1987 he was referred to the skin clinic. He complained of intense itching and even though he had on two pullovers and an anorak in a centrally heated out-patient clinic he complained bitterly of the cold. He was reluctant to undress for examination. A detailed history was difficult because of a confused mental state. Examination revealed that the whole skin was weeping and there were widespread excoriations. There was marked diffuse hair loss and generalized lymphadenopathy, and there was gross pitting oedema. His temperature in the clinic was 35·2°C. His weight was 68 kg, an increase of 12 kg from that recorded 6 months earlier. Investigation showed an Hb of 9·3 g%, compared to 13 g% in April; the blood film revealed a marked eosinophilia (2800/mm^3). The serum albumin had fallen over the preceding 9 months from 45 to 28 g/l but liver function tests were normal. A skin biopsy showed features of a chronic dermatitis, and a lymph node biopsy showed changes of dermatopathy only. Ultrasound examination showed no abnormality of the liver or spleen.

A diagnosis of exfoliative dermatitis of unknown cause was made and he was readmitted. In hospital he was started on prednisolone 40 mg daily with dramatic effect. His skin rapidly improved, and as it did so his symptoms of itching and of constantly feeling cold abated entirely. The lymphadenopathy disappeared and the abnormalities in his blood count, thyroid function tests and serum albumin resolved. His temperature returned to normal for the first time for nearly a year, and perhaps most remarkably his mental state was apparently transformed. He became alert and animated once again and happily returned to his lodgings, where there have been no further problems with his behaviour. Over a period of 3–4 months his hair regrew. His steroid dosage was gradually tailed down to 15 mg daily, and on this dosage he remains symptom free. There were no subsequent problems with hypothermia.

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Rosacea induced by PUVA therapy

Sir, The benefit from oral psoralen photochemotherapy (PUVA) may be offset by side-effects that can include exacerbation of coexistent light-sensitive skin disease such as SLE.[1] We report a case of rosacea presenting after a course of PUVA therapy, which to our knowledge has not been specifically described.

A 79-year-old Irishman presented with a 1-year history of a scaly erythematous rash over his body, which had gradually been becoming more extensive. On examination he had a typical rash of chronic plaque psoriasis, covering approximately 20% of the body area, including areas of the scalp but not the face.

He was started on PUVA treatment with 1 J/cm^2 of UVA three times a week for the first week but an inflammatory rash on the tip of the nose was noted and the dose reduced to 0·5 J/cm^2 three times a week. After a further week the dose was again increased to 1 J/cm^2 three times a week and he received a total of 11 J/cm^2 of UVA over 3·5 weeks. At the end of this course his psoriasis had cleared but a second rash had appeared on his face, starting on the nose and spreading in a symmetrical fashion on to the cheeks. The rash was erythematous, with rose red papules and isolated pustules. A biopsy was performed and the histology showed vascular ectasia, dermal oedema and some perivascular histiocytic infiltration with granulomata, consistent with the clinical diagnosis of rosacea. Extensive solar elastosis was also seen. Treatment with oxytetracycline, 250 mg twice daily produced clearance of the rosacea within 3 months, and the psoriasis did not recur.

Although ultraviolet light is not thought to be of primary importance in the pathogenesis of rosacea, the adverse effect of light is well known. Ultraviolet light may have a direct irritant effect and the production of prostaglandins inflammatory mediators may be harmful.[2] Excessive UV light may cause distortion of collagen structure and vascular damage, changes which may be found in rosacea patients, and the presence of solar elastosis in correlation with rosacea has been noted.[3] Among the immunological changes noted with rosacea is the presence of antinuclear factor,[4] levels of which are also raised after UV light exposure.[5]

Rosacea may be aggravated by natural sunlight and in our patient by PUVA therapy. Patients being considered for PUVA therapy should be examined for signs of rosacea and if present treatment should then only be given with caution.

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Complement deficiency in Sweet's syndrome

Sir, It has been suggested that complement may play an important role in the pathogenesis of Sweet's syndrome, but so far there is no definite evidence.[1–4] We have seen Sweet's syndrome in a patient with profound deficiency of the third component of complement (C3), an occurrence not previously recorded.

A 28-year-old Caucasian woman had a rash of 1 week's duration which had started on her legs and spread to her arms and neck. There was no history of recent febrile illness nor of medication and she had no other complaints. She had had recurrent infections since infancy that included pneumonia, meningococcal meningitis and otitis media.

Examination revealed multiple, tender, bluish-red plaques varying in diameter from one to several centimetres. The lesions were large and confluent on the backs of the arms and smaller and more scattered on the legs and V of the neck. There were no other abnormalities. A skin biopsy showed the histology of Sweet's syndrome and direct immunofluorescence of a frozen section of a second skin biopsy was negative for IgG, IgM, IgA, C3 and fibrin.

Laboratory investigations that included a full blood count showed a mild leucocytosis of $10{\cdot}5 \times 10^9$/l with 52% neutrophils, 42% lymphocytes, 5% monocytes and 1% eosinophils. The erythrocyte sedimentation rate was raised to 50 mm in 1 hour (Westergren). Antinuclear factor was negative. The amount of complement was measured by standard radial immunodiffusion with Endoplate agarose gels (Kallestad Laboratories, Inc. Austin, TX, U.S.A.). The value for C4 was 26 mg/dl (normal = 15–44 mg/dl) but no C3 could be detected. Previous immunological studies done when the patient was 15 years old[5,6] had shown the levels of C3 to be one-thousandth that of normal levels while the serum concentrations of C2, C4, C5, C6, CI inhibitor, C3 inactivator and properdin-factor-B were within normal limits.

The patient was treated with prednisone and the skin lesions responded promptly and cleared within 2 weeks. There has been no recurrence of the rash for over a year.

The aetiology of Sweet's syndrome is unknown but the frequent association with infections suggests that bacterial antigens or antigen–antibody complexes may play a pathogenetic role. Where Sweet's syndrome occurs in patients suffering from leukaemia, myeloma and solid tumours, antigens resulting from tissue break-down could play a role. The association with arthritis[7] and Sjogren's syndrome[8] and the prompt response to corticosteroids suggest that immunomechanisms are involved.

Immunological investigations have been performed in a limited number of patients and results have been inconsistent. Nunzi *et al.*[2] could not detect any circulating immune complexes and Going *et al.*[9] found no evidence of immune complex deposition in the skin. In some patients negative skin immunofluorescence for complement and immunoglobulins has been reported.[2,7–11] There have been single case reports of positive findings. IgA, IgM, C3 and fibrinogen were demonstrated in dermal vessels in one patient,[3] IgG, IgM and fibrin in a second[12] and IgD deposits in the dermis of a third.[9] In the cases in which serum complement has been measured, it has been either normal[7,11,13,14] or raised.[15]

Neutrophils predominate in the dermal infiltrate in Sweet's syndrome, suggesting that events resulting in chemotaxis play a significant role, however two studies have given conflicting results.[10] Some authors have speculated that complement plays a central role.[15,19] Others have suggested that Sweet's syndrome may represent a type 3 hypersensitivity reaction, which would of necessity result in complement activation.[3,4,16–18]

It is uncertain whether antigen-antibody complexes are capable of attracting neutrophils in the absence of complement. Observations by Alper *et al.*[6] suggest that although C3 or one of its fragments is involved in the mobilization of leucocytes, C3 may not be necessary for local chemotaxis *in vivo*. The absence of C3 in our patient provides evidence that complement-mediated polymorphonuclear chemotaxis does not play a role in Sweet's syndrome. Other mechanisms must therefore be responsible for the accumulation of neutrophils. Storer has suggested that substances such as kallikrein, plasminogen, immunoglobulin G and beta glucuronidase may stimulate chemotaxis in Sweet's syndrome[19] and a heat stable plasma chemoattractant for neutrophils has been isolated from a patient,[20] while Going[21] has recently suggested that interleukin I may play a central role.

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Book Reviews

Soft Tissue Sarcomas: Histological Diagnosis. (Biopsy Interpretation Series). A.D. NASH (1989). New York: Raven Press. Pp. 271. ISBN 0-89004-708-1. $81·50.

This small book is intended as a condensed guide to the diagnosis of soft tissue sarcomas for practising histopathologists. Each type of sarcoma is described in 11 chapters which are set out logically with sections describing the clinical features, gross and microscopic appearances and differential diagnosis. The final chapter, by Dr J.C. Vuletin, is a brief review of the ultrastructural appearances of sarcomas.

Most of the major diagnostic pitfalls are mentioned with those benign tumours and reactive conditions which may be confused with sarcomas, being discussed; although I was disappointed to see that proliferative fasciitis was not included. The references are adequate in that most major papers are cited, although few are after 1986. The lists are by no means comprehensive, and in particular few British or European papers are cited. The quality of many photomicrographs is disappointing with many low powers showing uneven illumination, little contrast, and a rather blurred appearance.

Dr Nash has succeeded admirably in summarizing a large volume of information into a remarkably small space. Unfortunately the book is a little too condensed to serve as a bench book. Although problems are discussed, the book lacks depth, and I have not found it of value in several difficult cases. Similarly the chapter on ultrastructure is a useful review but is of little value in other than straightforward cases.

Although not aimed as a competitor of *Soft Tissue Tumors* by Enzinger and Weiss (2nd edn 1988), prospective purchasers will want to compare the two. Nash is half the price, but less than a quarter of the size. Libraries will, I am sure, choose Enzinger and Weiss as the standard textbook, while diagnostic pathologists confronted with a problem will certainly want the larger heavily referenced and superbly illustrated book. Someone wishing to buy an introduction to the pathology of soft tissue sarcomas may well plump for the smaller and cheaper volume and will, I think, find it reasonable value.

R.REID

Surgical Anatomy of the Skin. S.J. SALASCHE, G. BERNSTEIN AND M. SENKARIK (1988). Norwalk, Connecticut: Appleton & Lange. Pp 269. ISBN 0-8385-8709-7.

Was the dissecting room really the happy place that sprang to mind as I perused the pages of this beautifully produced book? For the skin surgeon, as for any other, a thorough knowledge of relevant anatomy is a prerequisite and my experience is that it is very hard to refresh the memory from standard works.

This text is aimed at that increasingly common individual who recognizes the importance of surgical pursuits in the management of dermatological patients and who wishes to develop technical skills other than those traditionally taught in dermatology training programmes. Introductory chapters on surface anatomy, including one on aesthetics, are followed by a series of six chapters devoted to the classic systems of anatomy. Chapters on regional anatomy follow. Each chapter starts with a number of key features and ends with a discussion on clinical considerations. Throughout, the book is clearly illustrated with line drawings, diagrams and photographs of dissections and patients in a true amalgam of basic and clinical science.

My two concerns are relatively minor when taken against the overall quality but I was sorry to see that rather than references each chapter is followed only by a bibliography. Perhaps of more importance is the restriction of the contents to head and neck anatomy. Whilst I acknowledge this is the most important area in which we work there are important anatomic considerations at other sites which this book does not include.

This book is another indication of the importance that this branch of our speciality now has in the U.S.A. and reflects the quality of the leading proponents. It is yet another book for the training department's library and also one for firing the enthusiast who will find many useful tips among the clinical discussions.

N.P.J.WALKER

Skin Cancer. RONA M.MACKIE (1989). London: Martin Dunitz. Pp. 346, 435 illustrations. ISBN 0-948269-59-6. Price £60.00.

It is perhaps appropriate that I began my review of this book at the beginning of a short flight to Glasgow. In spite of this being a small, crowded plane, on a bumpy flight, I didn't put the book down until we landed. It is written in an easy style which encourages one to turn over the next page and continue on. It is particularly free from jargon, the views are expressed in a personal way which adds to the interest, of course, that is only what one would expect from the author and readers will not be disappointed. It contains a wealth of information with chapters on skin biology, aetiological factors in cutaneous malignancy, diagnosis and management, cancer-associated genodermatoses, and then nine chapters on tumours of various origins covering all the well known tumours and 'common rarities'. It was full of interesting pearls, for instance, rodent ulcers were first described by an Irishman in 1827; Alibert, while he was first to describe mycosis fungoides in 1806, had only seen one case; early palmo-plantar melanomas are specially deceptive in that they may look benign on clinical examination; it is important to examine the whole body because 5% of patients with melanoma have multiple lesions; accurate clinical diagnosis is probably no more than 50%, emphasizing the need for biopsy of any lesions, no matter how doubtful.

As would be expected there is a very fine chapter on melanoma, which I would recommend to all dermatologists. Having read the chapter it has clarified my views about management of pigmented conditions, be they congenital small naevi, dysplastic naevi, or lesions which are doubtful. Of course one might say that while I have a clear view of it, it is one person's view but knowing the amount of thought, study and research which the author has put into the subject, I am happy to stick with this view, until, as Damon Runyon said 'a better one comes along'.

The book is aimed primarily at dermatologists, with a good clinical section and enough pathology to satisfy most, or, at the very least, to whet the appetite. It is the sort of book to turn to when one meets an odd lesion and you feel you have seen it somewhere before, because the various tumours are beautifully illustrated by clinical photographs with good photomicrographs. I found myself succumbing to the temptation to look ahead to see if I could diagnose the illustrated lesions myself. I found it a good self audit. I never had any difficulty in seeing what the photograph was meant to illustrate in the legend. I couldn't say that for every book I have reviewed.

I recommend every dermatological department to have one, I believe it would also be useful to pathologists, particularly those who report biopsies but perhaps do not have a lot of clinical contact for it would give them a very clear picture, both by photograph and in the text, of the tumour they were reporting.

The text is up-to-date and I believe the book will be around for some time before a second edition is necessary.

D.BURROWS

Books Received

Acne and Related Diseases. Edited by R.MARKS AND G.PLEWIG (1988). London: Martin Dunitz. ISBN 0-948269-94-4.

British Journal of Dermatology (1989) **121**, 419.

News and Notices

The Urticarias—1990
Robinson College, Cambridge, 8–9 January 1990

There have been major advances in understanding of the molecular and clinical aspects of the urticarias and angio-oedema in recent years. Following the success of the 1984 international meeting in Cambridge, we propose to hold a second symposium in which an international faculty will discuss recent advances in this field. This symposium will be held in Robinson College, Cambridge, 8–9 January 1990, organized jointly by Professor M.W.Greaves and Dr A.Kobza Black from the Institute of Dermatology, London and by Dr R.H.Champion and Dr R.J.Pye from Addenbrooke's Hospital, Cambridge. Further information can be obtained from: The Conference Secretary, Postgraduate Medical Centre, Addenbrooke's Hospital, Cambridge or from the organizers.

13th Annual Meeting of the Association for Dermatologic Surgery
Vienna 18–20 May 1990

A meeting will be held in Vienna of the Association for Dermatologic Surgery and the topic is that of new and proven techniques in Dermatologic Surgery. Further information can be obtained from Wiener Medizinische Akademie für arztliche Fortbildung und Forschung, Alser Strasse 4, A-1090 Wien, Austria.

Fourth Combined Skin Pathology Course, Denver Colorado, 11–15 October 1989

This is a vigorous, comprehensive, practical review and update for dermatologists, pathologists, and dermatopathologists that is approved for 36 hours of CME I credit.

Microscopes and extensive syllabus material will be provided. There will be 44 formal lectures, four supervised microscope sessions of 700 outstanding workshop slides (20 h) and optional independent study.

For further information, please contact James E.Fitzpatrick, Dermatology Service, Fitzsimons Army Medical Center, Aurora, Colorado 80045, U.S.A. (1-303-361-8647).

MRC Programme Grant—Institute of Dermatology

An MRC programme grant has been awarded to Dr Richard Camp and Professor Malcolm Greaves at the Institute of Dermatology for research on the molecular basis of leucocyte infiltration and keratinocyte activation in inflammatory skin disease, with effect from 1 July 1989. The total value of the grant is £540 000.

Royal Society of Medicine

Dr Arthur Rook has been elected an Honorary Member of the Royal Society of Medicine.

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Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin

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SUMMARY

The immunocytochemical identification and characterization of indigenous dermal dendritic cells (dermal dendrocytes) using a rabbit polyclonal antibody to clotting enzyme factor XIII subunit A (FXIIIa) was carried out on normal and inflamed human cutaneous tissue. The immunophenotype of FXIIIa positive dendritic cells was analysed with a panel of 18 monoclonal antibodies using immunoperoxidase and double immunofluorescence staining techniques.

The antibody against FXIIIa detected highly dendritic dermal cells located particularly in the upper reticular and papillary dermis. Double fluorescence microscopy showed that FXIIIa positive cells were bone marrow derived (HLe-1$^+$) and co-expressed monocyte, macrophage or antigen presenting cell markers (HLA-DR$^+$, LFA-1$^+$, HLA-DQ$^+$, OKM5$^+$, Mo 1$^+$, Mono-1$^+$, Leu M3$^+$). No labelling was obtained with cell markers for Langerhans cells (CD1), T lymphocytes (CD2), granulocytes (LeuM1) fibroblasts (Te7), intercellular adhesion molecule-1 (ICAM-1) or endothelial cells (Factor VIII related antigen).

Gamma interferon induced increased expression of HLA-DR and co-expression of ICAM-1 on FXIIIa$^+$ dermal dendritic cells in normal skin in organ culture. Moreover, in benign inflammatory dermatoses such as atopic eczema and psoriasis there was an increased number of FXIIIa$^+$, DR$^+$, ICAM-1$^+$ cells in the upper dermis and foci of FXIIIa$^+$ cells in the epidermis closely associated with lymphocytes.

FXIIIa positive cells in human skin represent a specific population of bone-marrow dermal dendritic cells, distinct from Langerhans cells, that share some features common to mononuclear phagocytes (monocyte/macrophages). In addition, the detection of HLA-DQ on 48% of FXIIIa$^+$ cells and the lack of OKM1 in combination with high OKM5 expression suggests an antigen-presenting cell phenotype.

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There is increasing phenotypic and functional evidence for the recognition of several dendritic antigen-presenting cells (APC) which are both lymphoid and non-lymphoid associated. Well characterized lymphoid APCs include dendritic reticulum cells, interdigitating dendritic cells[1] and follicular dendritic cells.[2] Circulating immunocompetent dendritic cells have also been found in human peripheral blood[3,4] and afferent lymph.[5] Non-lymphoid immunocompetent dendritic cells include Langerhans cells,[6] collagen associated dendritic cells[7,8] as well as dendritic cells in the synovium,[9] respiratory tract,[10] thyroid gland[11] and heart.[12] It has been suggested that macrophages and dendritic cells have a common origin in the human yolk sac but diverge early in foetal development.[13] Activation and proliferation of antigen-specific effector T cells are dependent upon recognition of antigens by APC which bear class II major histocompatibility complex (MHC) antigens. These can be expressed on both epidermal and dermal cells including Langerhans cells,[6] endothelial cells,[14] B cells,[15] melanophages,[16-18] as well as dermal[19,20] and other dendritic cells.[3,21,22]

The derivation and role of the DR$^+$ dermal dendritic cell is still uncertain. Using enzyme histochemical and immunochemical techniques it has been shown that many dermal dendritic cells share features with mononuclear phagocytic cells (macrophages) and have a phagocytic function.[8] In addition, as in humans, class II MHC bearing dermal cells distinct from endothelial cells are present in mouse skin, and function as APCs *in vitro* and initiate primary contact hypersensitivity responses to contact allergens.[19,20]

Recently, there has been renewed interest in the cytoplasmic identification of the subunit A of the clotting proenzyme factor XIII (FXIIIa). The protein is expressed in histiocytic reticulum cells (sinus lining cells) of lymphoid tissue,[23] peripheral blood monocytes,[24-26] peritoneal macrophages,[27] reactive fibroblasts,[28] and recently in dermal dendritic cells.[29] Nemes *et al.*[30] regard this protein as a marker of phagocytic function.

The aim of this study was to further characterize FXIIIa positive dendritic cells in normal and inflamed human cutaneous tissue using immunocytochemical techniques. We have investigated the lineage of FXIIIa positive dermal dendritic cells including their relationship to Langerhans cells and monocyte/macrophages, and also whether these cells could be stimulated by gamma interferon (IFN-γ) to express intercellular adhesion molecule-1 (ICAM-1) or CD1. We also looked at the role of FXIIIa$^+$ cells in inflammatory dermatoses.

METHODS

Skin biopsies

Six normal neonatal foreskins were obtained at routine circumcision. Punch biopsies of skin (6 mm) were performed on three healthy volunteers and on lesional skin from patients with chronic plaque-type psoriasis (8) and atopic eczema (10).

Specimens from both foreskin and adult skin biopsies were divided into 2-mm portions. One portion was embedded in gum tragacanth (Sigma Chemical Co., St Louis, MO, U.S.A.), mounted on cork and frozen in isopentane, cooled in liquid nitrogen, and stored, wrapped in aluminium foil at −70°C until use. Other divided portions were incubated in 5 ml of Dulbecco's modified Eagle's medium (Gibco Laboratories, Grand Island, NY, U.S.A.) supplemented with 10% heat-inactivated fetal calf serum (Gibco Laboratories) either alone or with recombinant IFN-γ (Dr M. Shephard, Genentech Inc., San Francisco, CA, U.S.A.) (specific activation—1·7 × 10 U/mg, normal concentration of 1200 μ/ml). Incubated specimens were maintained with periodic agitation in a humidified incubator with 5% CO_2/95% air at 37°C for 48 h, then embedded as described above.

TABLE 1. Antibodies directed towards human mononuclear cells

Antibodies	Specificity
Polyclonal	
Anti FXIIIa§§	
Anti FVIII related antigen (ra)	Endothelial cells¶¶
Monoclonal	
HLe-1	All bone-marrow derived cells*
Anti HLA-DR/Leu10	Class II MHC protein antigen (DR/DQ)*
Leu6	Cortical thymocytes Langerhans cells (CD1)*
LFA-1	Lymphocyte function associate antigen-1 (Lymphocyte/monocytes)†
Leu5b	T cells (CD2)*
LeuM1	Myelomonocytes*
LeuM3	Monocyte/macrophage antigen (CD14)*
LeuM5	Monocyte/macrophage antigen*
Avidin	Mast cells*
Anti FVIII ra	Endothelial cells‡
OKM1	Monocyte antigen (C3bi receptor)§
OKM5	Monocyte and platelet antigen§
Mo 1	Myeloid antigen or monocytes, granulocytes and null cells‡
Mo 2	Myeloid antigen or monocytes and platelets‡
Mono-1	Monocyte/macrophage antigen¶
RFD1	Interdigitating, reticulum cells**
Te7	Fibroblasts††
RR-1/1	Intercellular adhesion molecule-1 (ICAM-1)‡‡

Source.
* Beckton Dickinson, Sunnyvale, CA, U.S.A.
† Dr C.Clayberger, Stanford University.
‡ Coulter Immunology, Hialeah, FL, U.S.A.
§ Ortho Immunodiagnostics, Raritan, NJ, U.S.A.
¶ Bethesda Laboratories/Gibco, Gaithersburg, MD, U.S.A.
** Dr L.Poulter, Royal Free Hospital, London, U.K.
†† Dr B.Haynes, Duke University, Durham, NC, U.S.A.
‡‡ Dr T.Springer, Dana-Faber Cancer Institute.
§§ Behringwerke, Marburg, F.R.G.
¶¶ Atlantic Antibodies, Scarborough, ME, U.S.A.

Double immunofluorescence labelling

Five micron cryostat sections were cut and air-dried. A panel of antibodies to various human mononuclear cell types was employed in an indirect immunofluorescence labelling technique (Table 1). Skin sections were incubated with primary antibodies for 45 min followed by three 5-min washes in phosphate-buffered saline (PBS). Second-step staining was performed with the use of goat anti-mouse IgG (1/40) (heavy- and light-chain-specific-affinity purified, human-serum absorbed and fluorescein conjugated; Kirkegaard and Perry, Gaithersburg, MD, U.S.A.) for 30 min. Labelling for detection of polyclonal rabbit anti-FXIIIa or factor VIII (FVIII) related antigen (1/40) and mouse monoclonal antibodies (1/20) was accomplished using rhodamine on fluorescein-conjugated goat anti-rabbit IgG (1/20) (Tago Inc., Burligame, CA, U.S.A.) in a similar manner. Endothelial cells were visualized with a directly fluorescein-conjugated goat anti-human factor VIII related antigen (Atlantic Antibodies, Scarborough, ME, U.S.A.).

Double-labelling immunofluorescence microscopy was performed by visualizing bound antibody against FXIIIa with fluorescein-conjugated F(ab)2 goat anti-rabbit IgG (Tago Inc.) followed by mouse monoclonal antibody and normal rabbit IgG to block cross-reactivity of the conjugated antibodies; the mouse antibody then being visualized with rhodamine-conjugated goat anti-mouse IgG. (Cappel Laboratories, Cockranville, PA, U.S.A.). Control sections were incubated in normal rabbit serum instead of primary polyclonal antibody and in mouse serum instead of monoclonal antibodies to verify the absence of xerogenic cross reactivity of the conjugated antibodies. Mast cells were visualized using avidin-conjugated to fluorescein. All dilutions were made up in PBS with 10% fetal calf serum.

Evaluation of fluorescence was performed with a Nikon microscope equipped with epifluorescence and appropriate filters. Identical fields were photographed and the percentage of each cell marker was analysed against labelling with FXIIIa.

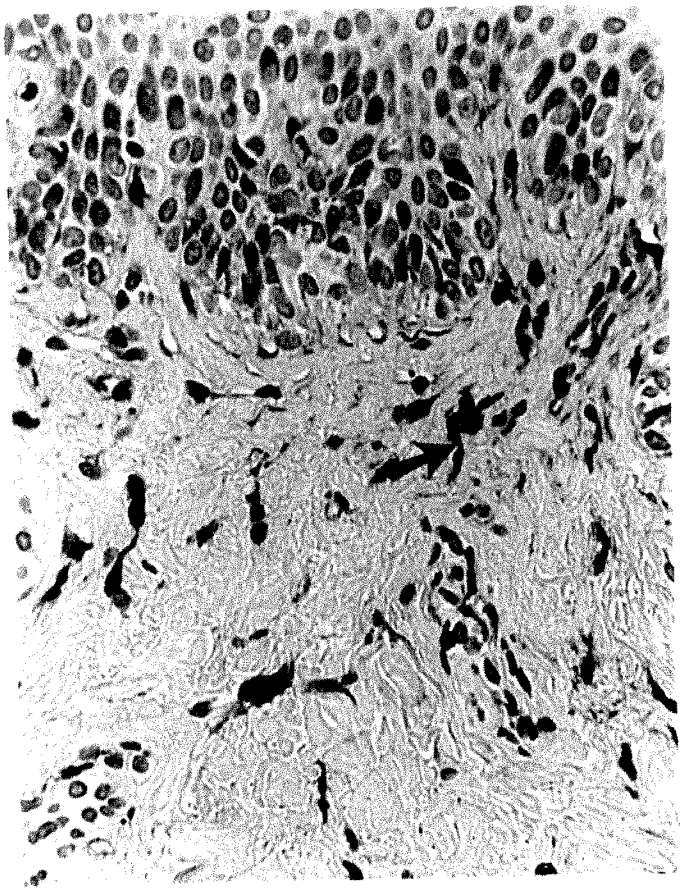
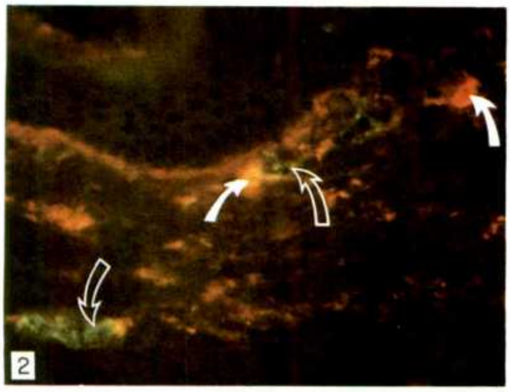
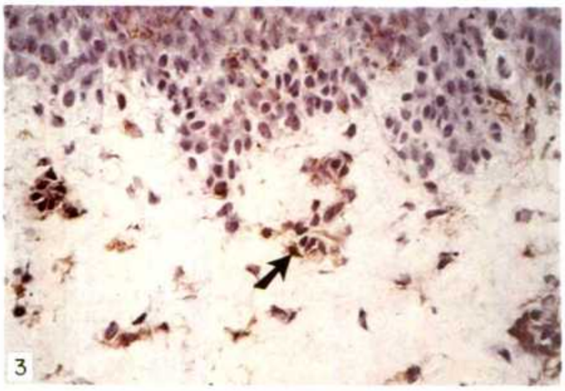
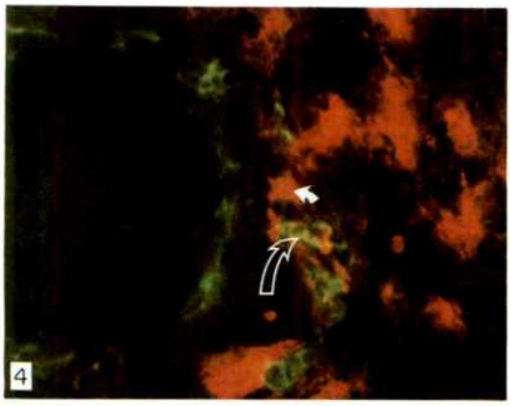


FIGURE 1. Factor XIIIa positive dendritic cells within the dermal papillae (arrow) in normal skin (× 300).







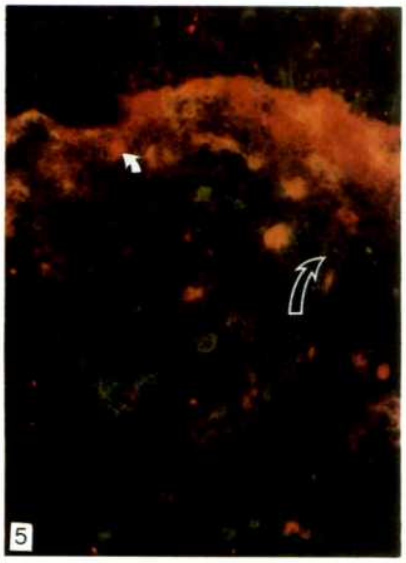


FIGURE 2. Negative double-indirect immunofluorescence labelling with FXIIIa (rhodamine, closed arrow) and FVIII related antigen (fluorescein, open arrow) in normal skin (× 150).

FIGURE 3. HLe immunoperoxidase labelling of dermal dendritic cells (arrow) in normal skin (× 150).

FIGURE 4. Negative double immunofluorescence labelling with FXIIIa (rhodamine, closed arrow) and Leu6 (fluorescein, open arrow) in normal skin (× 180).

FIGURE 5. Positive double immunofluorescence labelling (shown in yellow/orange) with FXIIIa (rhodamine, closed arrow) and HLA-DR (fluorescein, open arrow) in normal skin (× 180).

Immunoperoxidase labelling

Fresh frozen cryostat sections (5 μm) were cut and dried and fixed in acetone at 4°C for 10 min. Staining was accomplished with an avidin–biotin peroxidase technique (Vectastain ABC mouse kit—Vector Labs., Inc., Burlingame, CA, U.S.A.) employing the monoclonal antibodies listed in Table 1. The optimal concentrations of these monoclonal antibodies were determined to provide maximal specific labelling with minimal non-specific or background staining (data not shown). Either 3,3′-diaminobenzidene tetrahydrochloride or 3-amino-9-ethycarbazole were used as the chromogen. The sections were counterstained in 1% haematoxylin. FXIIIa was also identified in routine paraffin-processed specimens. Sections were de-waxed in xylene, followed by rehydration in graded alcohols. Subsequently, slides were trypsinized (trypsin [Sigma, St. Louis, MO, U.S.A.] 0·1% in 0·1% $CaCl_2$ in distilled water, pH 7·8 for 10 min at 37°C) and treated with 0·5% H_2O_2 in absolute methanol for 30 min to block endogenous peroxidase activity. The sections were then labelled in the same way as cryostat specimens employing a Vectastain ABC rabbit kit. The primary FXIIIa rabbit antibody was applied at a dilution of 1/200–1/400.

RESULTS

Normal skin

$FXIIIa^+$ dermal dendritic cells could be demonstrated in both neonatal foreskin and adult skin. They were distributed mainly in the upper dermis but especially in the papillary dermis around superficial blood vessels (Fig. 1). No labelling was seen in the epidermis in normal skin, nor in adnexal structures or neural tissue.

To determine the derivation of $FXIIIa^+$ cells in the dermis, double immunofluorescence labelling was carried out in both neonatal and adult skin with similar results. FXIIIa was not expressed by endothelial cells expressing FVIII related antigen (Fig. 2), or by fibroblasts expressing Te7 or by mast cells visualized by avidin-binding. However, 87% of $FXIIIa^+$ cells expressed the bone marrow derivation marker HLe-1. This was supported by HLe-1 immunoperoxidase labelling of dermal dendritic cells (Fig. 3). Among bone marrow-derived cells $FXIIIa^+$ cells failed to express T lymphocyte (CD2) or Langerhans cell (CD1) markers (Fig. 4), but 85% did express HLA-DR (Fig. 5), LFA-1 and 48% HLA-DQ, suggesting a

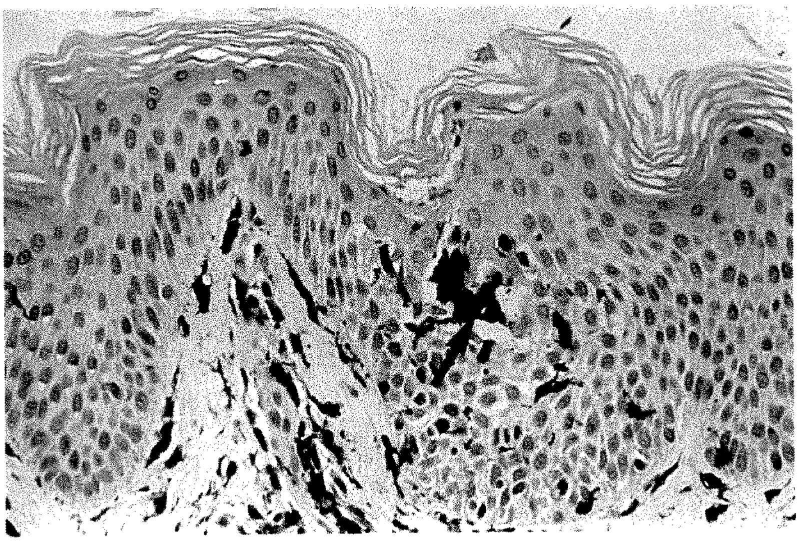


FIGURE 6. Immunoperoxidase labelling of dendritic cells (arrow) in abnormal epidermal location with polyclonal antibody FXIIIa in atopic eczema (×320).

monocyte/macrophage and/or dendritic APC lineage. The majority of FXIIIa$^+$ cells expressed some monocyte/macrophage antigens, i.e. 92% OKM5$^+$, 88% Mo 1$^+$, 92% Mono-1$^+$ and 22% Leu M3$^+$ but lacked Leu M5, Leu M1 and Mo2 expression. The detection of HLA-DQ on 48% of FXIIIa$^+$ cells and the lack of OKM1 in combination with high OKM5 expression suggests an APC phenotype. Unstimulated FXIIIa$^+$ cells did not co-express RFD-1 or ICAM-1.

OKM5 is also expressed on some FVIII related antigen-positive endothelial cells in skin,[18] raising the possibility of overlap between FXIIIa$^+$, OKM5$^+$ cells and FVIII$^+$ endothelial cells. However, not all OKM5$^+$ cells expressed FXIIIa and none of the FVIII$^+$ endothelial cells were identified as positive for FXIIIa, indicating that OKM5 is expressed on at least two separate types of dermal cells.

These findings were confirmed using immunoperoxidase single-labelling techniques in cryostat sections of normal skin.

Inflamed skin

FXIIIa labelling of skin from benign inflammatory dermatoses such as atopic eczema and psoriasis compared to normal skin revealed an increase in the number of dermal dendritic cells. Numerous FXIIIa dendritic cells were demonstrated around superficial blood vessels particularly in atopic eczema (Fig. 6). In contrast to normal skin, foci of FXIIIa$^+$ dendritic cells were observed in the epidermis associated with lymphocytes, especially where spongiosis and vesicle formation were most evident (Fig. 7). In skin from patients with chronic plaque psoriasis the increase in FXIIIa$^+$ cells appeared to be confined to the papillary dermis, and in two cases dendritic processes from FXIIIa$^+$ cells were observed traversing the basement membrane into the epidermis, closely associated with inflammatory cells (Fig. 8). Individual positively labelled cells were also seen in the epidermis which appeared to originate from the dilated papillary blood vessels.

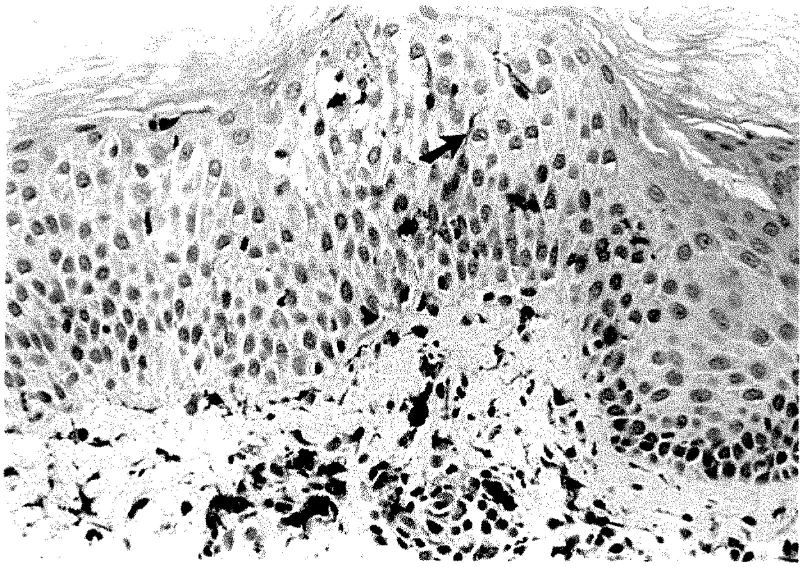


FIGURE 7. FXIIIa immunoperoxidase labelling of dendritic cells (arrow) in atopic eczema (× 320).

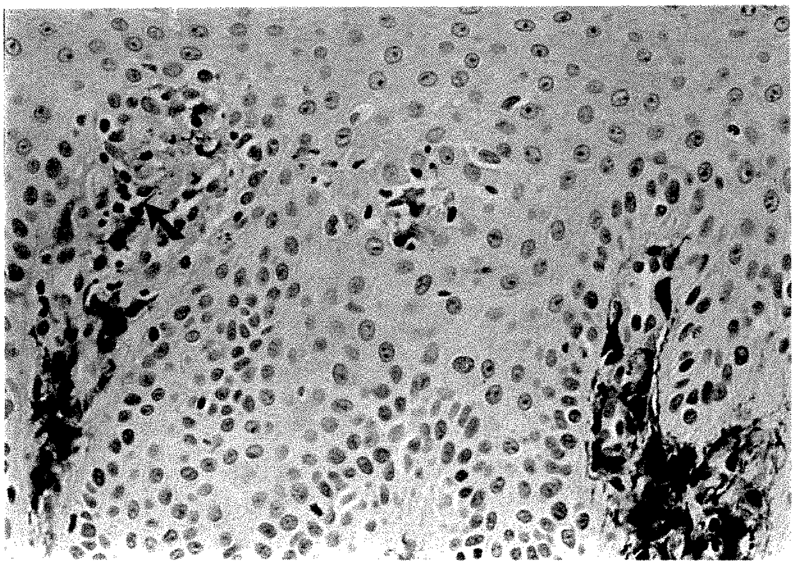


FIGURE 8. Immunoperoxidase labelling of dendritic cells (arrow) with FXIIIa in psoriasis (× 320).

In normal neonatal foreskin and adult skin in the modified organ culture system, ICAM-1 expression was confined to endothelial cells but when stimulated with IFN-γ, ICAM-1 was found to be induced on FXIIIa$^+$ dermal dendritic cells. Furthermore, there appeared to be increased expression of HLA-DR on these cells, although this was not quantified.

DISCUSSION

The findings in this study confirm the previous suggestion that FXIIIa is a cell marker for dermal dendrocytes,[29,31] and provide phenotypic and functional evidence for the characterization of a bone marrow-derived HLA-DR$^+$ dermal dendritic cell population, distinct from Langerhans cells. In contrast to Langerhans cells they lack CD1 and have been shown to be phagocytic.[31] Unlike previous reports[28] we have shown that in cutaneous tissue, FXIIIa$^+$ cells are not fibroblasts (i.e. Te7$^-$) which agrees with the recent report of negative FXIIIa labelling in fibrocollagenous cutaneous disorders.[29] FXIIIa$^+$ cells are bone marrow-derived cells with some of the cell surface antigens found on monocyte/macrophage subsets (OKM5$^+$, Mo 1$^+$, Mono 1$^+$, Leu M3$^+$).

We suggest that FXIIIa$^+$ OKM5$^+$ dermal dendritic cells comprise a pluripotential population, capable under various circumstances of macrophage effector functions,[16-18] APC functions,[19,21,32,33] and perhaps even differentiation into Langerhans cells upon migration into the epidermis.[34] Recently, Weiss *et al.*[18] have shown that dermal phagocytic cells, containing melanin, in inflammatory skin diseases demonstrate the surface phenotype of CD1$^-$DR$^+$, OKM5$^+$, OKM1$^-$, APC's and activated macrophages (Mo3$^+$). These OKM5$^+$ dermal dendritic melanophages may represent the same subtype as detected by FXIIIa and may derive from them. Moreover, $CD1^-DR^+$, $OKM5^+$, $OKM1^-$ melanophages have been demonstrated in human epidermis after ultraviolet light exposure and have been shown to have potent APC activity.[16] The $FXIIIa^+$ dermal dendritic cells appear distinct however, in that they express Leu M3 and Mo1, whereas $CD1^-DR^+$ $OKM5^+$ epidermal melanophages lack these two cell surface antigens.[16] Similarly, increased $CD1^-DR^+$ subpopulations of epidermal cells have been demonstrated in psoriasis[35,36] and recently, other inflammatory dermatoses.[37,38] Ultrastructural studies have suggested that at least some of the $CD1^-DR^+$ epidermal dendritic cells were of the Langerhans cell lineage. Our studies have shown that $FXIIIa^+$ dermal dendritic cells can be induced by IFN-γ to co-express ICAM-1. In inflamed skin such as atopic eczema and psoriasis, $FXIIIa^+$ cells appear to migrate from the papillary dermis into the epidermis in close proximity to lymphocytes and other inflammatory cells, probably by virtue of their ICAM-1 positivity. We propose that some of the $CD1^-DR^+$ epidermal dendritic cells are $FXIIIa^+$. In UV-induced inflammation the $CD1^-DR^+$, $OKM5^+$, $OKM1^-$ melanophages exhibited the capacity to present antigen and to activate autologous T cells in the absence of added antigens.[16] Our immunophenotypic characterization suggests that $FXIIIa^+$ dermal dendritic cells may represent the cutaneous analogue of potent blood APC, described as a minor subset of monocytes with a phenotype $OKM5^+$ $OKM1^-$.[32] These cells may be responsible in a similar way for stimulating immunoregulatory T cells that modulate other immune responses.[38–39] If $FXIIIa^+$, Class II MHC bearing dermal dendritic cells are analogous to murine Class II MHC dermal dendritic cells, then they would be expected to process and present soluble and alloantigens to T cells[17] and to be able to initiate allergic contact hypersensitivity responses.[20]

Expression of the antigen detected by OKM5 has also been shown on endothelial cells[16] suggesting that FXIIIa might also be identified in endothelial cells. However, double staining studies with antifactor VIII related antigen showed that as with $OKM5^+$ melanophages, these bone marrow derived cells are distinct from endothelial cells.[18]

Functional studies with the modified organ skin culture[41] showed that when stimulated by IFN-γ, FXIIIa positive dermal cells demonstrate increased expression of ICAM-1. The latter is an inducible glycoprotein found on vascular endothelial cells, mitogen-stimulated T lymphocytes, EBV transformed B cells and tissue macrophages[42] and is important for leucocyte trafficking into tissues.[43] ICAM-1 is the ligand for lymphocyte function associated antigen-1 (LFA-1) found on lymphocytes and monocytes. The expression of ICAM-1 by the dermal dendrocyte may facilitate adhesive interactions with T lymphocytes as we have proposed for keratinocyte lymphocyte reaction.[41,43] We have recently labelled fixed and unfixed cultured human fibroblasts for LFA-1 and FXIIIa and there was no cell surface immunolabelling. Also, fibroblasts are LFA-3 positive but neither LFA-1 nor FXIIIa could be induced on fibroblasts with either IFN-γ or IFN-α.

In conclusion, $FXIIIa^+$ dendritic cells in normal skin and in some examples of inflammatory cutaneous diseases represent a specific immunological cell population with multiple potential functional properties as defined by their immunophenotype. Moreover, cytoplasmic FXIIIa can be detected in routine paraffin-embedded sections allowing superior preservation of morphological detail which permits a retrospective study of this cell marker in a wide spectrum of cutaneous diseases. There is evidence to support the hypothesis that dermal dendritic cells, or important subsets thereof, constitute an immunologically competent cell population which is indigenous to the dermis if not to all collagen-rich tissues.[29,31] It would be reasonable for dermal immunologic competence to be complementary to epidermal immunologic function, particularly in a wide variety of immunologically mediated inflammatory dermatoses. Recently, we have reported that FXIIIa$^+$ dermal dendritic cells are increased in a wide variety of inflammatory, fibrohistiocytic and granulomatous dermatoses including AIDS-associated Kaposi's sarcoma also suggesting that these cells have an important role in the pathophysiology of these disorders.[44,45]

ACKNOWLEDGMENTS

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Localization of foetal antigen 2 (FA-2) in foetal and adult human skin

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SUMMARY

Foetal antigen 2 (FA-2) is a connective-tissue-associated antigen isolated from second trimester human amniotic fluid. FA-2 has an α-electrophoretic mobility and is a single-chain molecule with a molecular weight of 26 kDa as determined by polyacrylamide gel electrophoresis (PAGE). Using indirect immunofluorescence and the immunoperoxidase technique, FA-2 was found to be in the lamina densa/sublamina densa region of the basement membrane zone (BMZ) in adult as well as in foetal skin. FA-2 was found throughout the dermis in foetal skin, whereas in adult skin it was found to be associated with the BMZ and around the blood vessels, hair follicles and eccrine glands. Intracellular FA-2 antigen was demonstrated in proliferating fibroblasts by the indirect immunoperoxidase technique and immunoelectron microscopy of the fibroblasts revealed staining of the antigen in the cisternae of the rough endoplasmatic reticulum at the trans-side of the Golgi complex as well as in vesicles close to the plasma membranes. FA-2, a hitherto undescribed antigen associated with human BMZ, is probably being synthesized by proliferating fibroblasts.

Antibodies against endometrial proteins have been generated using fractionated second trimester human amniotic fluids (AF) as the antigen source.[1] In the same study antibodies were produced against two hitherto undescribed antigens present in AF and the foetal circulation. The distribution of these two antigens suggested a foetal origin and they were referred to as foetal antigens FA-1 and FA-2, respectively. It was shown that the antigens were immunologically distinct from α-fetoprotein, another foetal antigen present at a high concentration in AF.

Following production of monospecific antibodies against the two antigens, the indirect immunoperoxidase technique was used to study the distribution of FA-1 and FA-2 in foetal tissues.[2] It was shown that FA-1 was exclusively present in the foetal hepatocytes, whereas FA-2 was present in foetal mesenchymal tissues, with condensed staining corresponding to the basement membranes. This study was undertaken to further characterize FA-2, to examine its distribution in foetal and adult human skin and to study the site of synthesis of the antigen.

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METHODS

Immunoreagents

Monospecific rabbit antibodies against human foetal antigens (FA-1 and FA-2) were produced using fractions of second trimester amniotic fluid as previously described.[1] Rabbit anti-laminin anti-serum was donated by Dr R.Albrechtsen, University Institute of Pathological Anatomy, Copenhagen University. Normal rabbit IgG and peroxidase-conjugated swine anti-rabbit IgG (Dakopatts, Copenhagen, Denmark) and alkaline phosphatase-(AP) conjugated goat anti-rabbit IgG (Sigma) were used. In the analysis of the specificity of the rabbit anti-FA-2, antibodies against α-feto-protein (AFP; Dakopatts) and against FA-1, placental protein 12 (PP-12) and PP-14[1] were used.

Skin samples

Foetal skin specimens were obtained from three foetuses with gestational ages of 7, 8 and 10 weeks. Punch biopsies were obtained from three healthy volunteers without skin disease. Skin was obtained with a keratome from three fresh cadavers without any skin disease and used for the preparation of NaCl-separated skin. The foetal, normal and cadaver skin specimens were rinsed in phosphate-buffered saline (PBS, pH 7·2). Specimens of 0·25 cm^2 were mounted in OCT compound (Miles Scientific), snap frozen in CO_2 and stored at −20°C. The split skin technique was used as previously described[3,4] with modifications.[5]

Electroimmunoassays

All electroimmunoassays, i.e. crossed immunoelectrophoresis (XIE), line immunoelectrophoresis (LIE) and rocket immunoelectrophoresis (RIE) were performed in 1·5 mm thick 1% (w/v) agarose in 0·02 M Tris-barbital buffer (pH 8·6), the same buffer being used as electrophoresis buffer. The electrophoreses were run at 25 V/cm for 18 h except for the first dimensional run of XIE which was performed at 10 V/cm, using bromphenol-blue stained albumin as migration marker.

Cell cultures

Human embryonal lung (HEL), human skin (HS) and murine embryo (ME) fibroblast cultures were kindly donated by Dr S.Mogensen, Institute of Medical Microbiology, Århus University, Denmark. Five culture plates of each cell type were investigated.

Indirect immunofluorescence and immunoperoxidase technique

Indirect immunofluorescence and indirect immunoperoxidase staining were performed on 5 μm cryostat sections of foetal skin, normal skin, cadaver skin and NaCl separated skin, as previously described.[5,6] For immunoperoxidase staining of HEL, HS and ME fibroblasts, monolayer cultures were washed three times in RPMI-1640 and the monolayers were fixed in 4% paraformaldehyde for 10 min at 20°C.[2]

Immunoelectronmicroscopy

The fibroblast cell cultures were fixed in 0·1% (v/v) glutaraldehyde, buffered to pH 7·2 with 0·1 M cacodylate. The fixative solution was supplemented with 0·05% (w/v) saponin. The cultures were fixed at 4°C for 30 min. The indirect immunoperoxidase staining with anti FA-2 and anti-FA-1 was performed on cell cultures, as previously described.[7] After the chromogenic reaction the cultures were fixed in 2·5% (v/v) glutaraldehyde in 0·1 M phosphate buffer at room temperature for 1 h, and after rinsing in PBS, postfixed in 1% (w/v) OsO_4 in 0·1 M phosphate buffer at room temperature for 1 h. All cultures were prestained with 1% (w/v) uranyl acetate in redistilled H_2O for 1 h, then dehydrated in ethanol and embedded in Araldite®. After polymerization selected areas with a monolayer of fibroblasts were cut out of the bottom of the culture flasks. Semi- and ultra-thin sections were cut along planes parallel to the bottom of the flasks. Ultra-thin sections, contrasted with 0·4% (w/v) lead citrate for 5 min at room temperature, were examined in a Jeol JEM 100CX at 80 kV.

Purification of FA-2

Amniotic fluid (AF) from the second trimester was obtained and FA-2 antigen was purified from AF as previously described.[1]

Polyacrylamide gel electrophoresis

Samples of AF and FA-2 were diluted in sample buffer [10% (v/v) glycerol, 3% (w/v) sodium-dodecyl-sulphate (SDS), 12·5% (v/v) Tris-buffered saline, 48% (w/v) urea, 0·1% (v/v) bromphenol-blue] and boiled for 2 min with and without addition of 0·6 M dithiothretiol (DTT). Alkylation was achieved by adding 1·4 M iodoacetamide to the reduced (DTT) samples and 0·02 M iodoacetamide to the unreduced samples. Proteins in the AF and FA-2 samples were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on homogenous methylene-bis acrylamide slab gels, using a 4% stacking gel with a 7% separating gel, at 40 mA per gel.

Prestained proteins were included as molecular weight markers with molecular weights ranging from 26–180 kDa (Sigma, cat. no MW SDS-BLUE).

Immunoblotting

Following SDS-PAGE the proteins were electrophoretically transferred to nitrocellulose paper. The free protein binding sites were saturated using Tris-buffered saline (TBS) (pH 7·4), containing 0·1% Tween-20. Strips of nitrocellulose paper were incubated with either rabbit anti-FA-2, rabbit anti-laminin or with normal rabbit IgG diluted in washing-buffer (PBS containing 0·05% Tween) or in washing-buffer alone for 4 h at room temperature. After five washings in washing buffer the strips were incubated with AP-conjugated goat anti-rabbit IgG (diluted 1:1000 in washing buffer) for 1 h. After washing the strips were developed with 10% (v/v) nitrobluetetrazolium (NBT), 1% (v/v) 5-bromo-4-chloroindoxyl phosphate (BCP), 0·002% (v/v) $MgCl_2$ in 0·1 M ethalonamine buffer (pH 9·0) for 30 min at room temperature.

RESULTS

Characterization of antibodies and molecular weight determination of FA-2

The specificity of the rabbit anti-FA-2 antibodies was analysed by crossed immunoelectrophoresis (XIE) and line immunoelectrophoresis (LIE). In XIE with a concentration of anti-FA-2 of 75 $\mu l/cm^2$ gel (i.e. 1:2 dilution) only one precipitation arc in the electrophoretic α_1 region was seen, when 15 μl AF was applied, whereas with 15 μl normal human serum as the antigen source no precipitate was seen. The analysis of anti-FA-2 in a pentaspecific LIE system is shown in Figure 1. The pentaspecific antibody was directed against antigens present in AF, i.e. α-fetoprotein (AFP), placental protein 12 (PP-12), PP-14, FA-1 and FA-2. Negative deflections of the line precipitate indicates presence of antibodies with specificity against the corresponding antigens. It is seen from Figure 1 that the anti-FA-2 used in the present study did not react with other antigens isolated from AF, i.e. AFP, PP-12, PP-14 and FA-1. FA-2 antigen appeared in

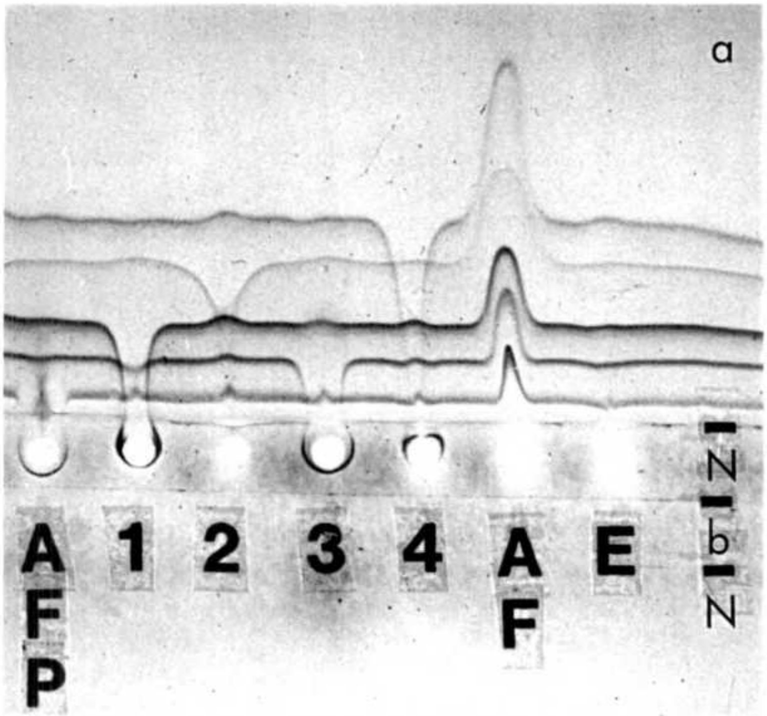

FIGURE 1. Line immunoelectrophoresis using a mixture of five antibodies with specificities against PP-12, PP-14, FA-1, FA-2 and AFP in the upper antibody gel (a). The line gel (b) contained 2·5 μl AF/cm² gel. N = neutral gels. The wells contained anti-AFP (AFP), anti-PP-12 (1), anti-PP-14 (2), anti-FA-1 (3), anti-FA-2 (4) and amniotic fluid (AF) and no antibodies (E). Negative (downwards) deflection of a line precipitate indicates the presence of antibodies with specificity against the corresponding antigen in the well. It is seen that the anti FA-2 antibodies used in (4) exclusively deflect the FA-2 line.

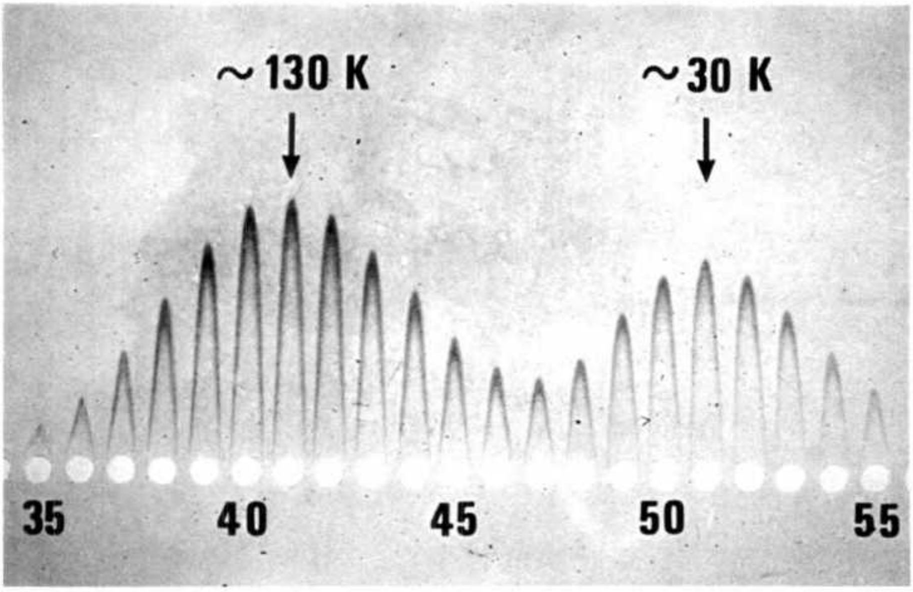

FIGURE 2. Rocket immunoelectrophoresis against anti-FA-2 of fractions eluted from Sephacryl S-200 column.

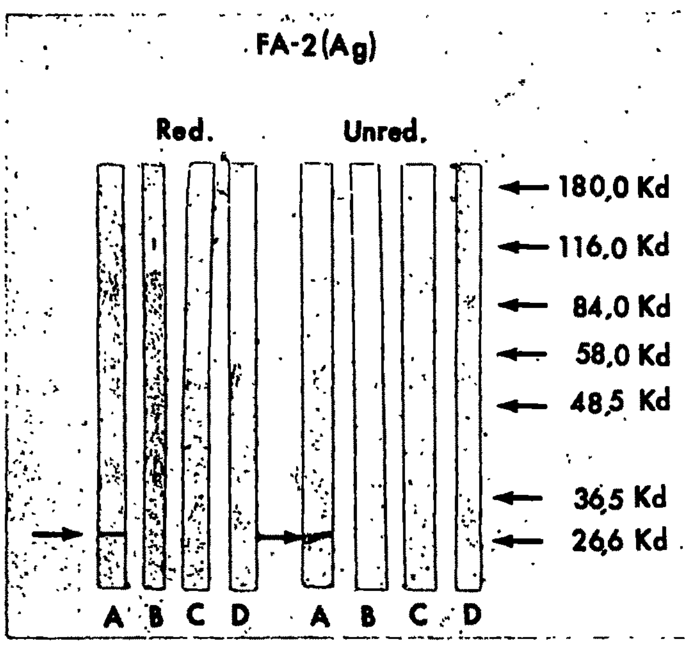

FIGURE 3. Immunoblot analysis of purified FA-2 antigen under reducing (left) and unreducing (right) conditions. Strips were incubated with: anti-FA-2 (A), anti-laminin (B), normal rabbit IgG (C) and buffer (D) and subsequently AP-conjugated goat anti-rabbit IgG.

two distinct peaks following chromatography (Sephacryl S-200) with molecular weights around 30 kDa and 130 kDa, respectively (Fig. 2). However, analysis of the molecular weight of FA-2 by immunoblotting (Fig. 3) revealed only one molecular form with a molecular weight of 26 kDa. The molecular weight of 26 kDa was found for FA-2 in AF as well as for purified antigen, and no difference was seen between the reduced or unreduced form of antigen. These two molecular forms of FA-2 showed immunological identity when analysed by double immunodiffusion.

Localization of FA-2 in foetal and adult skin

On foetal skin anti-FA-2 produced a linear fluorescent band along the BMZ as well as diffuse fluorescence throughout the dermis (Fig. 4a). On normal adult skin anti-FA-2 produced a broad fluorescent band at the BMZ, extending into the adjacent dermis (Fig. 4b). Staining occurred around the blood vessels, hair follicles and eccrine glands, while the remaining connective tissue of the dermis was unstained. The same pattern was seen on cadaver skin. On split skin the fluorescence was located at the dermal side of the split (Fig. 4c). By contrast, anti-laminin on foetal skin as well as on adult skin produced a sharp fluorescence band at the BMZ (Fig. 4d and 4e), located at the dermal site (Fig. 4f). With anti-laminin antibodies, only perivascular fluorescence was seen in the dermis. The same localization of FA-2 as demonstrated by immunofluorescence was found by the indirect immunoperoxidase technique.

Demonstration of FA-2 in fibroblast cultures

Indirect immunoperoxidase staining with anti-FA-2 on monolayers of human skin fibroblasts showed intracytoplasmic and perinuclear staining (Fig. 5a). No staining was obtained with anti-

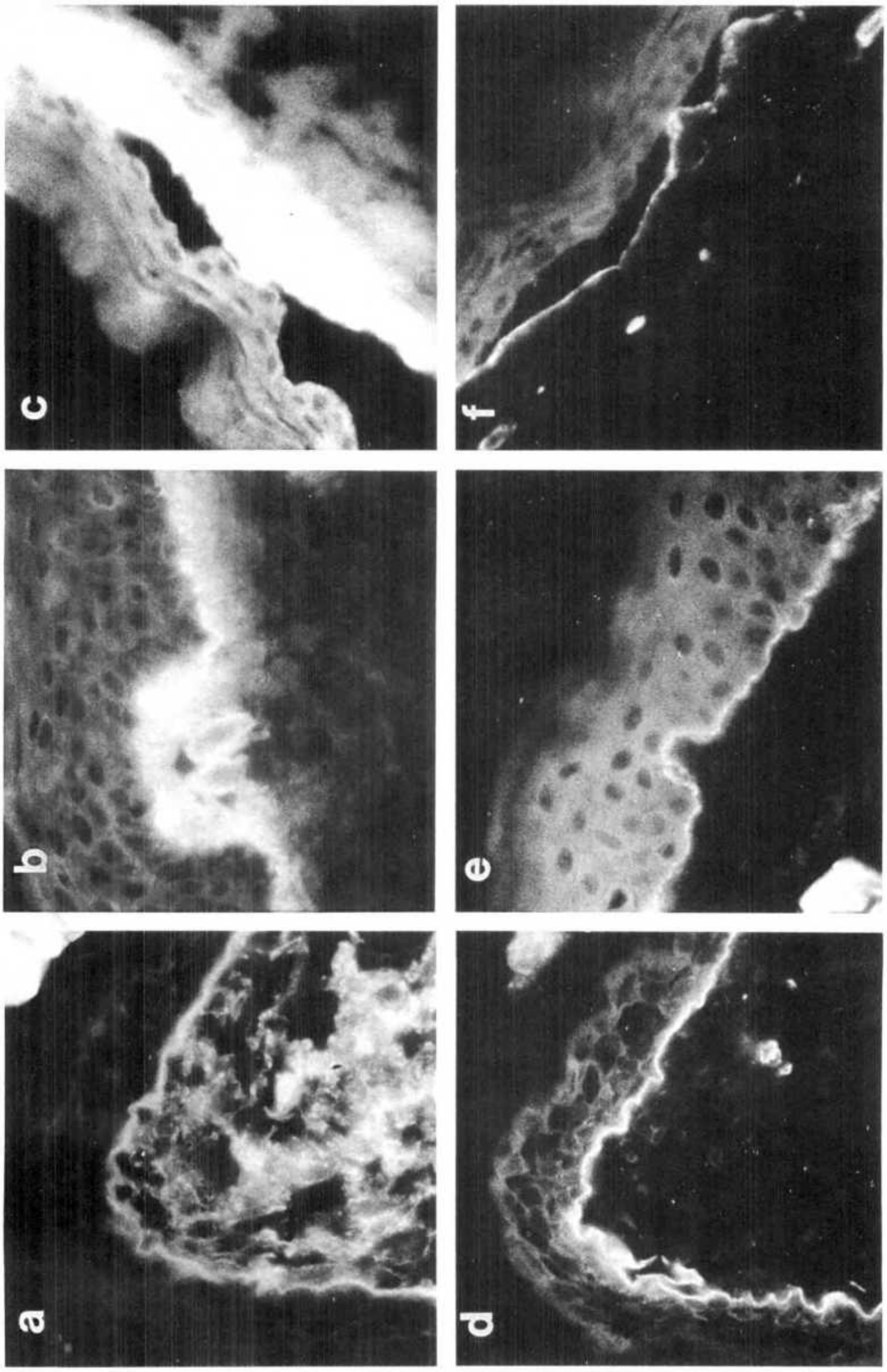

FIGURE 4. Indirect immunofluorescence with anti FA-2 (a–c) and anti-laminin antibodies (d–f) on: foetal skin (a and d), adult skin (b and e) and adult, NaCl-separated skin (c and f) (×288).

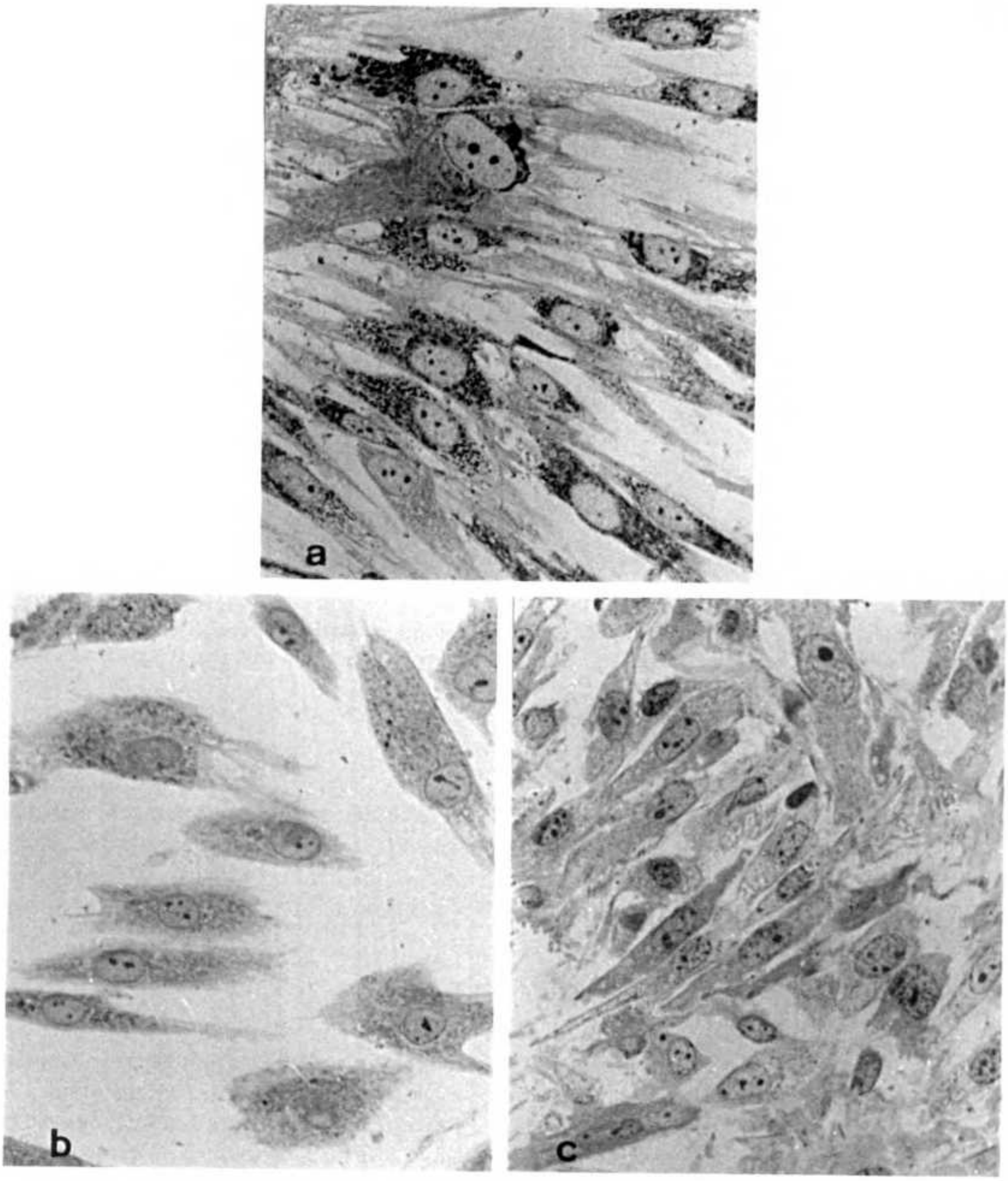

FIGURE 5. Indirect immunoperoxidase staining with (a) anti-FA-2 on human skin fibroblasts, (b) anti-FA-1 on human skin fibroblasts and (c) anti-FA-2 on mouse embryo fibroblasts (× 400).

FA-1 on human skin fibroblasts (Fig. 5b), and neither of the antibodies stained mouse embryo fibroblasts (Fig. 5c). The same staining pattern was seen with human embryo lung fibroblasts as with human skin fibroblasts.

The immunoreaction product involving FA-2 was shown by electron microscopy to be an amorphous electron dense substance (Fig. 6) and all cisternae of the rough endoplasmic

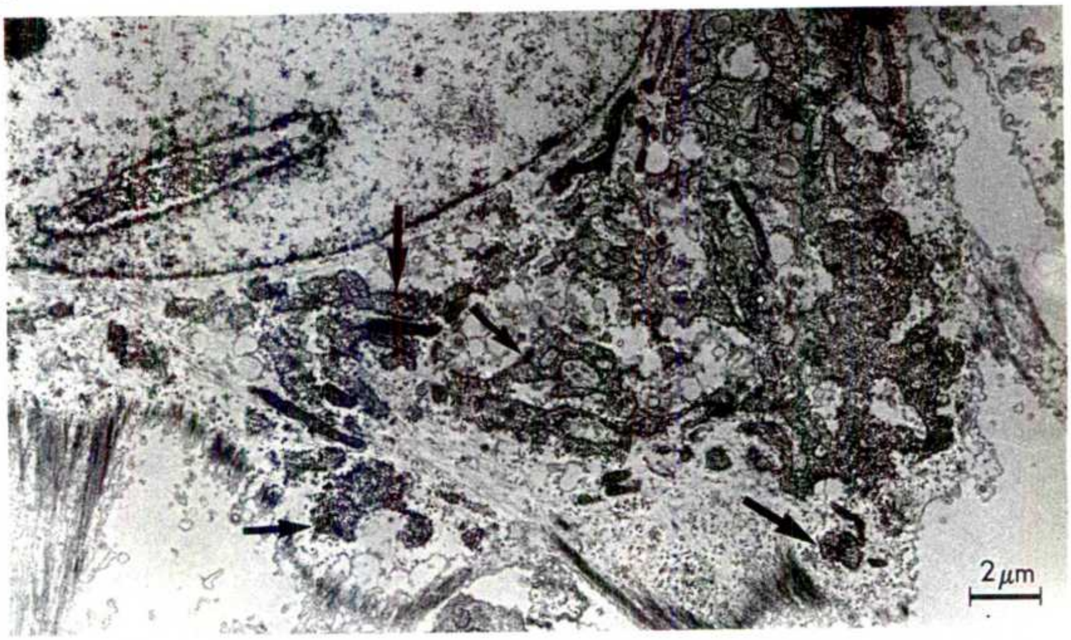

FIGURE 6. Immunoelectronmicroscopy of human skin fibroblasts with staining reaction for FA-2. A fine granular reaction product is seen in dilated cisternae of the rough endoplasmatic reticulum (arrows). Reaction product is absent at the cell surface (× 4420).

reticulum contained this reaction product. FA-2 was also detected in large vesicles, associated with the trans side of the Golgi complex and in vesicles close to the plasma membrane. Nuclei, mitochondria, microfilaments and microtubules were unstained. No obvious extracellular localization of FA-2 (cell surface and microfibrils) was found.

DISCUSSION

The IgG preparation against FA-2 used in the present study showed monospecificity; the antibody only produced one precipitate when analysed in crossed immunoelectrophoresis in high concentration (i.e. 75 $\mu l/cm^2$ agarose, 1 $cm^2 = 150\ \mu l$), and only when amniotic fluid was used as antigen source. When the same antibody preparation was analysed in LIE against five antigens present in high concentration in amniotic fluid (AFP, the endometrial proteins PP-12 and PP-14 and FA-1 and FA-2), the antibody reacted exclusively with one of the five precipitates. Analysis of the molecular weight of FA-2 by gel filtration chromatography confirmed the previously published observation of apparent molecular heterogeneity of this antigen with estimated molecular weights around 30 and 130 kDa. However a more accurate estimation by Western blot analysis revealed only one molecular weight for the FA-2 antigen (26 kDa) and this molecular weight was identical whether the PAGE was performed under reducing or non-reducing conditions. Thus, the FA-2 antigen seems to consist of a single chain, and the high molecular weight form (130 kDa) seen after gel filtration chromatography may be a simple aggregate which is dissolved in the presence of SDS.

In contrast to the staining with anti-laminin antibodies, an obvious difference between foetal and adult skin was observed with respect to localization of FA-2 antigen. In foetal skin FA-2 was present throughout the dermis with a condensation along the BMZ. In adult skin, however, FA-2 was present along the BMZ, in the adjacent dermis and around the blood vessels, hair follicles and eccrine glands, and was distributed in a more diffuse manner. Using the split-skin as substrate the antigen was found to be located at the lamina densa/sublamina densa region of the BMZ.[4,5]

The BMZ of the skin consists of a variety of collagenous and non-collagenous proteins, including glycoproteins and proteoglycans, located in specific zones in the BMZ.[8-11] Type IV collagen (molecular weight 560 kDa) is the major structural protein in the BMZ and is found in the lamina densa. It is important in epidermal adhesion and is synthesized by different cell types including keratinocytes. Laminin (molecular weight 900 kDa) is a glycoprotein found in the lamina lucida and has a role in the organization of basement membrane (BM) macromolecules and promotes the attachment of cells to the extracellular matrix. Heparan sulphate proteoglycan (molecular weight 750 kDa) is involved in BM organization and is the major constituent of the permeability barrier. The bullous pemphigoid (BP) antigen (molecular weight 220 kDa) has an intracellular localization associated with the attachment plaque of hemidesmosomes and occurs also in the lamina lucida. Type VII collagen (molecular weight > 1000 kDa), a newly described collagen, seems to be the filament structure of the anchoring fibrils in the sublamina densa zone. Chondroitin sulphate proteoglycan and entactin (molecular weight 150 kDa) are present in all BM and are located to the lamina densa. Monoclonal and polyclonal antibodies have helped to discover new antigens on unidentified BM proteins such as KF-1,[12] AF-1 and AF-2[13] which are located in the lamina densa and sublamina densa zone. AA-3 and GB-3[8] seem to recognize hemidesmosome components. The distribution and molecular weight of FA-2 suggest that this antigen differs from the above mentioned BMZ-constituents, and is distinct from BP[14] and the epidermolysis bullosa acquisita antigens.[15,16]

The difference in the localization of FA-2 between foetal and adult skin, and the diffuse fluorescence in the dermis of the foetal skin, lead to the hypothesis that the FA-2 antigen is synthesized by dermal fibroblasts which are actively proliferating during the development of the skin in the foetus. This is supported by the observation that FA-2 can be detected in the foetal circulation in the second and third trimester,[1] and by the demonstration of intracytoplasmic, perinuclear and granular staining of FA-2 in human fibroblast cultures. The presence of FA-2 on the rough endoplasmic reticulum, demonstrated by electron microscopy, is highly suggestive for this component being actively synthesized by these cells.

Fibronectin is also a connective tissue protein, synthesized by fibroblasts and located in the connective tissue adjacent to the BMZ in human skin. However, fibronectin is a dimer of two identical subunits of molecular weight 220 kDa, held together by disulphide bonds, whereas FA-2 is a single-chain molecule with molecular weight of 26 kDa. Also the anti FA-2 antibodies had been thoroughly absorbed with normal human serum (NHS), containing approximately 300 μg/ml of fibronectin, and did not react with any components in NHS by crossed immunoelectrophoresis.

In conclusion, we have identified a hitherto undescribed BMZ-associated antigen, which seems to be produced by proliferating fibroblasts. Whether FA-2 is a structural component of the BMZ or perhaps a factor with regulatory properties for proliferating epidermal cells remains to be elucidated. The possible involvement of the FA-2 antigen in pathological processes involving the BMZ is presently being studied.

ACKNOWLEDGMENTS

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Transonychial water loss: relation to sex, age and nail-plate thickness

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SUMMARY

The transonychial water loss (TOWL) was measured in 21 healthy volunteers with an evaporimeter, to establish the usefulness of this technique and study the influence of sex, age and nail-plate thickness. The median TOWL was 19·4 g/m^{2}h^{-1} and it decreased with increasing age ($R=0{\cdot}51, P<0{\cdot}018$). The median transepidermal water loss ($\text{TEWL}_{\text{HAND}}$) from the back of the hand was 16·0 g/m^2 h^{-1} and from the dorsal side of the underarm (TEWL_{ARM}) it was 5·6 g/m^{2}h^{-1}. The TEWL_{ARM} was significantly less than from any of the two other points studied ($P<0{\cdot}01$), while there was no significant difference between the values obtained on the hand and the nail. TEWL_{ARM} had a median value of 6·4 g/m^2 h^{-1} in men and 4·3 g/m^2 h^{-1} in women ($P<0{\cdot}05$) in this study. No significant correlation between nail-plate thickness, as measured by ultrasound 20 MHz A-scan, and TOWL was found. Measurements of TOWL are of interest and should be age-related. Further studies are needed to determine TOWL in various forms of nail pathology.

Brittle finger nails are a common complaint[1] and are often ascribed to changes in nail-plate hydration. A number of techniques have been devised to study the passage of water across the human nail *in vivo*. An evaporimeter convenient for the measurement of transepidermal water loss (TEWL) has been developed in Sweden by G.E. Nielsson[2] and has gained wide acceptance.

The present study was undertaken in order to evaluate the use of an evaporimeter (Servo Med EP1®) in the study of the physiological transonychial water loss (TOWL) of normal human nails, particularly in relation to nail thickness and TEWL elsewhere on the same limb. TOWL is of interest since neither the nail nor the nail bed has any sweat glands and this is an index for insensible water loss.[3].

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METHODS

The study was performed on 21 healthy volunteers (12 female, nine male), with no history of skin or nail disease and with no nail pathology on clinical examination. None used nail varnish at the time of examination and the volunteers had not immersed their hands in water for at least 10 min prior to the measurements. Their median age was 32 years (range 22–71 years).

The water loss was measured using an evaporimeter (Servo Med EP1®, Servo Med AB, Stockholm, Sweden).[2] The probe includes an open chamber 12 mm in diameter, mounted with sensors for the determination of temperature and relative humidity. The water loss was calculated automatically and expressed as g/m^2 h^{-1}. The reading was taken 30 s after the probe was applied, at which time the value was stable.

The water loss was studied in three places; the nail of the thumb (randomly left or right), the dorsum of the hand and the underarm. The thumb was chosen to have as large and as thick a nail as possible and the chamber pressed gently onto its surface so as to avoid any water vapour entering from the perinychium. Each measurement was repeated three times and the average value used.

After recording the TOWL, the TEWL$_{\text{HAND}}$ and the TEWL$_{\text{ARM}}$, the thickness of the nail plate was measured using high frequency (20 MHz) ultrasound A-scan (Dermascan A®, Cortex, Hadsund, Denmark).[4] For calculation of nail-plate thickness the acoustic velocity of 2459 m/s was used.[5] Nail thickness was expressed in mm. and all measurements were carried out in the same room of a comparable humidity and temperature.

For the statistical analysis Wilcoxon's paired and unpaired tests as well as Spearman's rank correlation test were used. The chosen level of significance was $P < 0{\cdot}05$.

RESULTS

TOWL, TEWL$_{\text{HAND}}$, TEWL$_{\text{ARM}}$, and nail-plate thickness are given in Table 1. The TOWL and TEWL$_{\text{HAND}}$ were found to be significantly larger than the TEWL$_{\text{ARM}}$ ($P < 0{\cdot}01$). No statistically significant difference was found between the TOWL and the TEWL$_{\text{HAND}}$.

Correlations were studied between age, TOWL, TEWL$_{\text{HAND}}$, TEWL$_{\text{ARM}}$, and nail thickness. A significant negative correlation ($R = 0{\cdot}51$, $P < 0{\cdot}018$) was found between age and TOWL (Fig. 1). No other significant correlation was found. No significant difference between TOWL in men and women could be shown.

TEWL$_{\text{ARM}}$ was 6·4 g/m^{2}h^{-1} (range 5·3–7·0) in men and 4·3 g/m^{2}h^{-1} (2·7–10·10) in women ($P < 0{\cdot}05$).

TABLE 1. Summarized results of the study

	Median value	Range
TOWL (g/m^{2}h^{-1})	19·4	11·7–33·5
TEWL$_{\text{HAND}}$ (g/m^{2}h^{-1})	16·0	6·2–34·4
TEWL$_{\text{ARM}}$ (g/m^{2}h^{-1})	5·6	2·7–10·1
Nail thickness (mm)	0·56	0·42–0·80

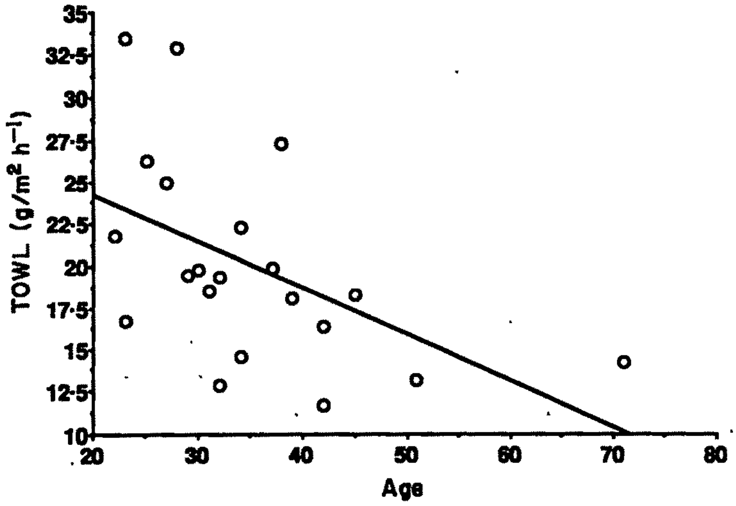

FIGURE 1. The negative correlation between age (years) and TOWL (g/m^2h^{-1}), $R = 0{\cdot}51$, $P < 0{\cdot}018$.

DISCUSSION

This study showed that the loss of water through the normal human nail plate was not significantly different from that of the dorsum of the hand (TEWL). It decreased with age and the sex of the individual was of no significance. The level of water loss appeared to be independent of the nail thickness.

In vitro studies have shown that the permeation patterns for the human nail differ significantly from those of normal human epidermis[6–9], allowing water to pass about 10 times faster through nail than through stratum corneum.[7] In spite of this better permeability the nail retains as much moisture as the stratum corneum, when studied at a moderate level of humidity.[10] This is reflected in the present study by the fact that the water loss from the nail was not found to be significantly different from that of the dorsum of the hand, although the former has no eccrine sweat glands.

Ultrasound and desiccation studies have shown the presence of a superficial water barrier in the human nail plate.[5] As the nail lacks a method of active water secretion, the TOWL can be regarded as an expression of the imperceptible water loss.[3] The lower levels of water loss found in our older subjects reflect the generally reduced hydration seen with increasing age.

The lack of correlation between the thickness of the nail and the TOWL *in vivo* has been reported previously in one person[11] using another type of equipment.

It is concluded that the evaporimeter (Servo Med EP1®) offers a simple and reliable method for the objective study of TOWL *in vivo*. Future studies should be carefully matched with respect to the age of the subjects.

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Changes in endothelial cell mass, luminal volume and capillary number in the gravitational syndrome

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SUMMARY

The histopathological changes in the skin of 31 patients with the gravitational syndrome and 10 normal control subjects were studied. To quantify the clinical severity of the syndrome, a new index of severity was used. Histometric evaluation of the cutaneous vasculature was performed on biopsies taken from the edge of the ulcers in the 31 patients and from the medial aspect of the lower legs in control subjects. Endothelial cell mass did not correlate with the clinical index of disease severity, suggesting that angiogenesis did not necessarily signal healing. The mean luminal volume appeared to be of greater prognostic value as there was a significant negative correlation of this parameter with the index of clinical severity. The degree of epidermal hyperplasia was found to be related to both the amount of inflammatory cell infiltrate present and the endothelial cell mass per unit tissue volume. The results also showed that the clinical index of disease severity was positively correlated to the mean epidermal thickness. The quantitative evaluation of biopsy material can be used to provide prognostic information in the gravitational syndrome.

Browse and Burnand[1] suggested that leakage of fibrinogen and the subsequent pericapillary polymerization to fibrin led to the development of a diffusion barrier to oxygen which in turn produced tissue hypoxia and ulceration. The same group,[2] using semi-quantitative methods for assessment of dermal vasculature, reported an increased thickness and number of dermal blood vessels in the skin from patients with the gravitational syndrome presenting with lipodermatosclerosis, when compared with normal controls. They found a good correlation between the degree of increase in the capillary bed and that of venous hypertension. This suggests that extensive neo-angiogenesis of the ulcerated site does not necessarily signify that healing will follow. This may be because the function of the new capillaries is inadequate or because the

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intra-tissue perfusion of oxygen is decreased on account of some other tissue disturbance independent of tissue vascularity. The purpose of this study was to further our understanding of the gravitational syndrome by determining the precise relationships between the structural parameters of capillary proliferation and the clinical status and outcome.

METHODS

Patients and controls

In this study 31 patients with ulceration of the lower leg (20 females, 11 males; mean age 70 ± 12 years, age range 43–90 years) were investigated. Patients were accepted if they satisfied the usual clinical criteria for a diagnosis of the gravitational syndrome and had clinical evidence of venous hypertension with no evidence of peripheral vascular disease, diabetes or other disorders which may cause or severely aggravate ulceration of the leg. In addition, 10 normal healthy control subjects without evidence of gravitational changes (seven females, three males; mean age 47 ± 7 years, age range 40–61 years), were also studied. All gave their informed and written consent to take part in the study. Ethical approval for the study was given by the Joint Ethics Committee of the South Glamorgan Area Health Authority and the University of Wales College of Medicine.

Assessment of disease severity

An arbitrary clinical scoring system was devised to quantify the overall severity of the disease process in each individual patient. The system was based on the clinical history and physical signs listed in Table 1, and the method by which a numerical value was given to each of these is outlined in Table 2. The scoring system is described in detail elsewhere.[3]

Histological preparation

Biopsies were obtained under 2% lignocaine anaesthesia using 6 mm diameter disposable trephines (Stiefel Laboratories Ltd., Slough, U.K.), from the superior margin of the ulcer in the gravitational syndrome patients and 5 cm above the medial malleolus in control subjects. After fixation in Bouin's solution for 24 h, tissue was alcohol dehydrated, cleared in cedarwood oil and

TABLE 1. Factors involved for scoring system in gravitational syndrome severity

Parameter	Maximum score possible
1 Duration of gravitational syndrome	3
2 Number of relapses	3
3 Total duration of ulcer present in all relapses	3
4 Duration of present ulcer	6
5 Depth of ulcer	3
6 Size of ulcer	5
7 Number of ulcers in both legs	3
8 Induration (surrounding skin)	4
9 Erythema (surrounding skin)	3
	33*

* Maximum total possible.

TABLE 2. Factors included in an arbitrarily derived clinical index of disease severity in gravitational syndrome patients.

Parameters		Score
1 Duration of syndrome	1–5 years	1
	6–10 years	2
	11–45 years	3
2 Number of relapses	No relapse	0
	1 relapse	1
	2 relapses	2
	Over 2 relapses	3
3 Total duration of ulcer present in all relapses	0 (no relapses)	0
	1–12 months	1
	1–2 years	2
	Over 2 years	3
4 Duration of present ulcer	1–6 months	1
	7–12 months	2
	1–2 years	3
	2–5 years	4
	5–10 years	5
	10 and more years	6
5 Depth of ulcer	Superficial	1
	Intermediate	2
	Deep	3
6 Size of ulcer	1–10 cm^2	1
	11–20 cm^2	2
	21–30 cm^2	3
	31–80 cm^2	4
	81 cm^2 and above	5
7 Number of ulcers in both legs	1 ulcer	1
	2 ulcers	2
	3 ulcers and above	3
8 Induration	No induration	0
	1–10 cm	1
	11–20 cm	2
	21–30 cm	3
	31–50 cm	4
9 Erythema	No erythema	0
	1–15 cm	1
	16–30 cm	2
	31–50 cm	3

embedded in paraffin wax. Serial sections, nominally 5 μm thick, were mounted five per slide and alternate slides stained with haematoxylin and eosin and periodic acid Schiff haematoxylin.

Histometric estimation of blood vessels

The histological tissue was subsampled using a systematic selection of slide number, section number and field number, all previously generated using random numbers. In all, eight fields

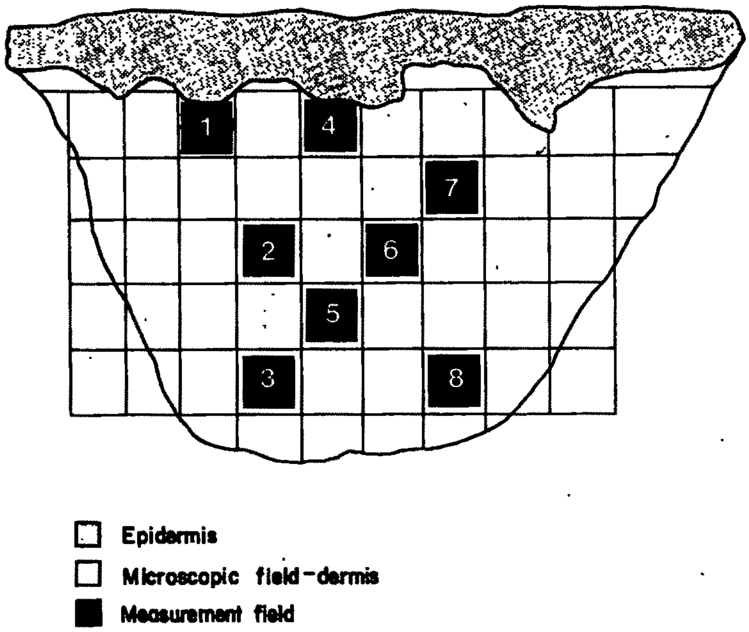

FIGURE 1. Diagrammatic representation of a histological section of the skin. Eight fields, selected in the systematic way shown above, were measured in six slides giving 48 fields in all from every biopsy.

from one section on each of six slides were assessed. Preliminary studies had shown that further measurements did not reduce the cumulative standard error[4] below 12% for endothelial volume, 17% for luminal volume and 10% for capillary number.

Microscopic fields, using × 40 objective lens magnification, were sampled as shown in Figure 1 and measurements of blood vessels performed by computer assisted planimetry (Hewlett Packard 85/Summagraphic digitiser). Cursor movement was viewed concurrently with the microscopic field via a camera lucida.

Tracing the outline of the outer wall of the blood vessel and subsequently the outline of the lumen allowed derivation of their respective areas with calibration and appropriate software (G.I.S. Ltd., Scotland). Normal counting procedures were used[5] to select those vessels within an area equivalent to 62 000 μm^2 delineated by an eyepiece graticule. Data from all 48 fields was used to derive the endothelial area (total vessel area − luminal area) and luminal area, both expressed per unit area dermis sampled. Under the Delesse principle this area fraction is proportional to the volume fraction.[5] The number of vessel sections per unit area was also derived.

Histometric assessment of the epidermis

From the same series of slides, using × 20 objective lens magnification, three fields on one section of six slides were assessed as follows. Measurements of the length of the stratum granulosum and the area of epidermis and the length of basal epidermis subtended by that length, were performed by computer assisted planimetry. Data from 18 fields was used to derive mean epidermal thickness (MET) (total epidermal area divided by total length along the stratum granulosum) and the ratio of the length of basal epidermis to the granular layer length (B/G ratio).

Assessment of dermal infiltrate

In histological samples from gravitational syndrome patients, further analysis was performed to estimate the degree of inflammatory cell infiltrate. This was carried out using a visual analogue scale, where each sample was assigned a point on a 100 mm line proportional to the observer's subjective estimate, zero being none and 100 being the maximum possible degree of dermal infiltrate.

Statistics

All histometric and clinical data were entered into the Minitab package[6] run by the South West Universities Regional Computing Centre. Morphometric data are ratios and thus unlikely to be normally distributed. Either non-parametric methods or arcsine transformation prior to parametric methods[7] were used in statistical hypothesis testing. The latter method was used to allow derivation of confidence intervals from the linear correlation of morphometric data with age of subject.[8]

RESULTS

In the 31 patients studied, the mean disease severity was 17·7 with a range from 11 to 27. These results are reported in full elsewhere.[3]

The results of histometric estimation of blood vessels of patients with gravitational ulcers and normal healthy control subjects are shown in Table 3. Biopsies were assessed for vascular endothelial volume, luminal volume and number of capillaries per unit dermal tissue volume (Fig. 2). All histometric data are expressed per unit dermal tissue volume × 10^{-3}.

TABLE 3. Results of histometric estimation of blood vessel parameters of 31 patients with gravitational ulceration and 10 normal healthy control subjects. Biopsies were assessed for vascular endothelial volume (VEV), vascular luminal volume (VLV) and number of capillaries (NV) per unit tissue volume using computer assisted planimetry. All histometric data are expressed per unit dermal tissue volume × 10^{-3}

		Ulcer present	Normal control
VEV	Median	50·00	23·65
	Mean	51·52	23·61
	SD	21·90	5·35
	n	31	10
VLV	Median	5·10	3·00
	Mean	5·91	3·07
	SD	3·98	1·16
	n	31	10
NV	Median	2·00	1·42
	Mean	2·29	1·32
	SD	0·90	0·30
	n	31	10

Statistical analysis (Mann–Whitney *U*-test)

VEV	Ulcer present vs. control	$w = 796$	$P < 0{\cdot}001$.
VLV	Ulcer present vs. control	$w = 721$	$P < 0{\cdot}05$.
NV	Ulcer present vs. control	$w = 759$	$P < 0{\cdot}01$.

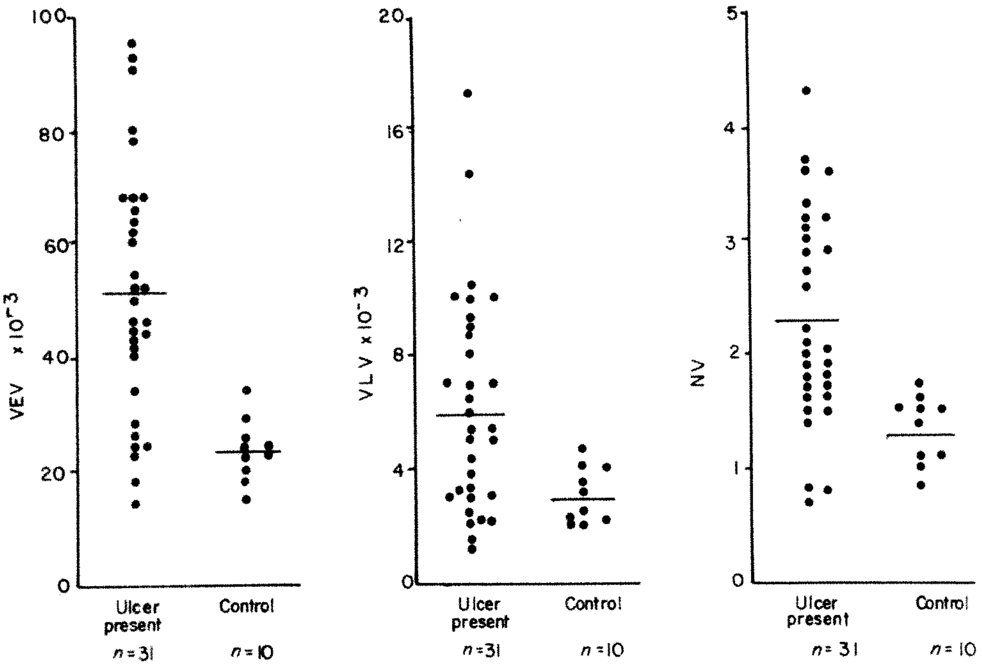

FIGURE 2. Results of histometric estimation of blood vessels of 31 patients with gravitational ulceration and 10 normal healthy control subjects. Biopsies were assessed for vascular endothelial volume (VEV), luminal volume (VLV) and number of capillaries per unit tissue volume (NV) using computer assisted planimetry. The mean values are shown by horizontal bars.

TABLE 4. Results of histometric estimation of blood vessels of patients with gravitational ulceration and 10 normal healthy control subjects. The ratio of vascular endothelial volume (VEV) to number of capillaries (NV) per unit tissue volume (NEV:NV ratio) and the ratio of vascular luminal area (VLV) to number of capillaries per unit tissue volume (VLV:NV ratio) were assessed for each patient and control

		Ulcer present	Normal control
VEV:NV ratio	Median	23·23	16·69
	Mean	22·98	18·81
	SD	6·01	7·95
	n	31	10
VLV:NV ratio	Median	2·09	2·12
	Mean	2·87	2·43
	SD	2·17	1·22
	n	31	10

Statistical analysis (Unpaired *t*-test after arcsine transformation)

VEV:NV ratio	Ulcer present vs. control	$t = 1{\cdot}95$	$P = 0{\cdot}06$.
VLV:NV ratio	Ulcer present vs. control	$t = 0{\cdot}43$	$P = 0{\cdot}67$.

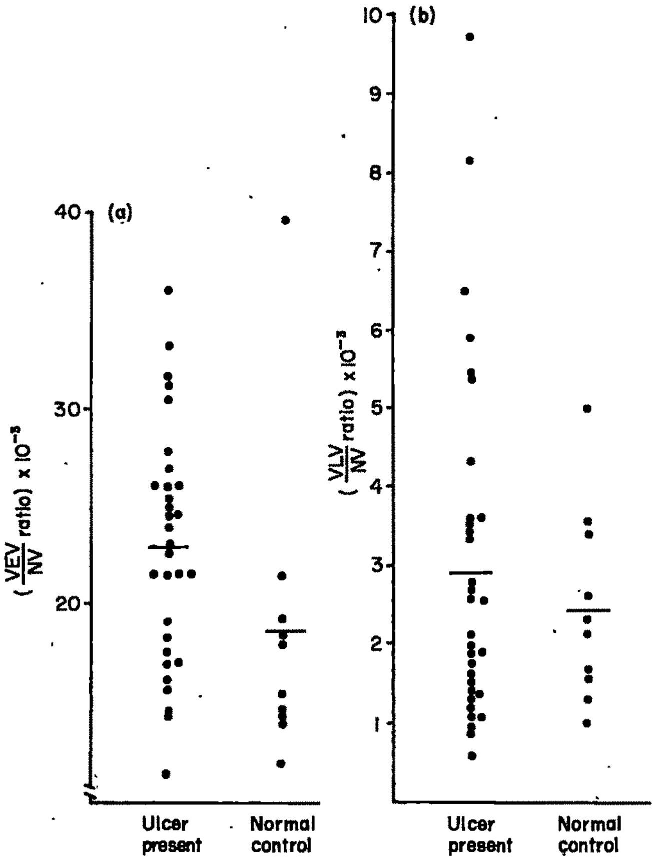

FIGURE 3. Results of histometric estimation of the blood vessels in 31 patients with gravitational ulceration and 10 normal healthy control subjects. (a) showing the ratio of vascular endothelial volume to number of capillaries per unit tissue volume (VEV:NV ratio). (b) showing the ratio of vascular luminal volume to number of capillaries per unit tissue volume (VLV:NV ratio). The mean values are shown as horizontal bars.

There was a significant difference between the endothelial volume in biopsies from gravitational syndrome patients as compared to controls (Mann–Whitney *U*-test, $P < 0.001$). The mean vascular endothelial volume in patients was 51.52×10^{-3} compared to 23.61×10^{-3} in control subjects. The results also revealed a significant difference between patients and controls in respect of their mean vascular luminal volume: 5.91×10^{-3} compared to 3.07×10^{-3} (Mann–Whitney *U*-test, $P < 0.05$). A further significant difference found was in the mean number of capillaries between gravitational syndrome patients and control subjects: 2·29 compared to 1·32 (Mann–Whitney *U*-test, $P < 0.01$).

However, to ascertain whether the increased vascular endothelial volume (VEV) and luminal volume (VLV) in gravitational syndrome patients compared to the control subjects was the result of the increased number of blood vessels (NV) in the dermis, the data was analyzed further. The mean value for VEV:NV was greater in gravitational syndrome patients than controls. The difference between the VLV:NV means was not so obvious (Table 4). Statistical analysis of arcsine transformed data did not support the hypothesis that these ratios differed

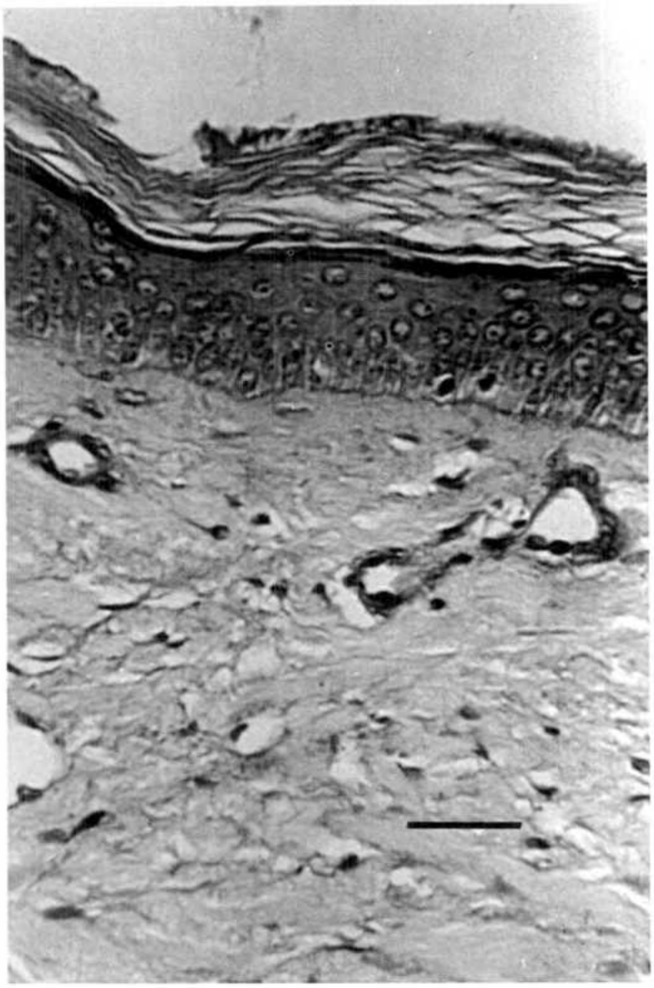

FIGURE 4. Skin blood vessels in the lower leg of normal healthy control subject (PAS-haematoxylin stain, bar = 25 μm).

significantly. Figure 3 shows the spread of VEV : NV and VLV : NV ratio data and in particular one very high VEV : NV result for the controls should be noted.

Photomicrographs of the dermis showing blood vessels in normal and gravitational ulceration biopsies are shown in Figures 4 and 5 respectively.

Table 5 and Figure 6 show the results of measurement of the mean epidermal thickness (MET) (μm) and basal : granular layer length ratio (B/G ratio) in gravitational syndrome patients and controls. Mean epidermal thickness (MET) is significantly different in patients compared to controls, being 314 μm compared to 57 μm (Mann–Whitney *U*-test, $P < 0{\cdot}001$).

Data from the two groups were separately plotted against age to determine whether any significant correlation could be derived. Table 6 shows the correlation coefficients together with their 95% confidence intervals. It can be seen that in all cases the coefficient is close to zero, indicating a lack of relationship for any of the morphometric parameters with age.

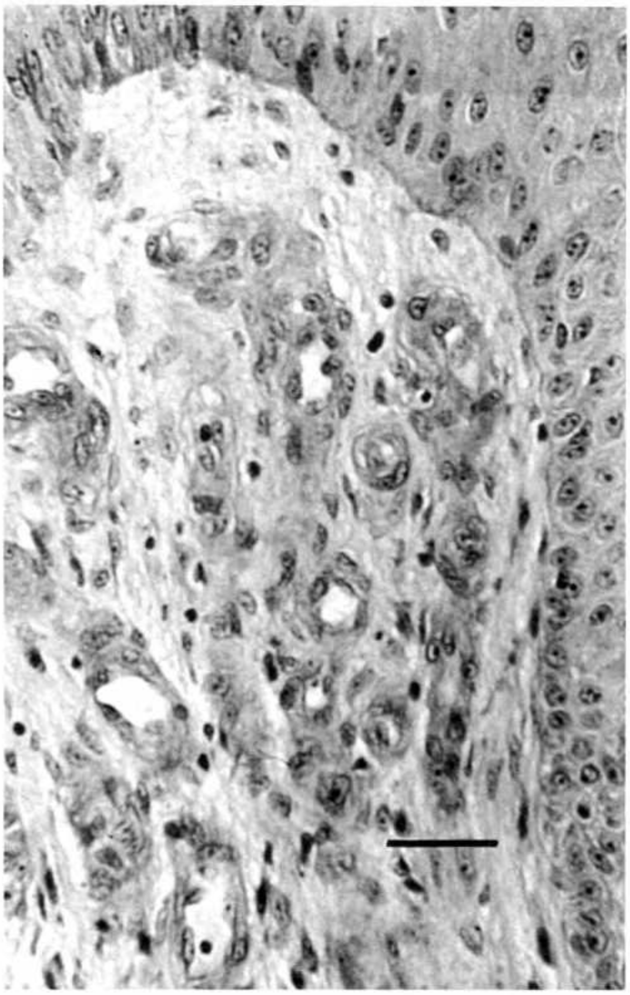

FIGURE 5. Thickened blood vessels in the skin from the edge of an ulcer in the gravitational syndrome. Numerous new small blood vessels can also be seen (PAS-haematoxylin stain, bar = 25 μm).

Correlation of histometric data with clinical status

Attempts to correlate the histometric parameters for blood vessels to the clinical index of disease severity showed that there was a statistically significant negative relationship between vascular luminal volume per unit tissue volume and the clinical index of disease severity (Fig. 7) (Spearman rank correlation, $R = -0{\cdot}365$, $P < 0{\cdot}05$). A positive relationship was found between all the histometric parameters for blood vessels and the extent of dermal inflammatory cell infiltrate. A positive relationship was also found between vascular endothelial volume per unit tissue volume and the mean epidermal thickness (Spearman rank correlation, $R = 0{\cdot}361$, $P < 0{\cdot}05$) (Fig. 8).

Mean epidermal thickness was also found to be related to the clinical index of disease severity (Fig. 9) (Spearman rank correlation, $R = 0{\cdot}359$, $P < 0{\cdot}05$).

TABLE 5. Measurement of the mean epidermal thickness (MET) and the basal : granular layer length ratio (B/G ratio) in 31 patients with gravitational ulceration and 10 normal healthy control subjects, assessed by computer assisted planimetry

		Ulcer present	Normal control
Mean epidermal thickness	Median	325·0	57·7
	Mean	314·5	57·0
	SD	70·0	6·99
	n	31	10
B/G ratio	Median	1·67	1·00
	Mean	1·74	1·00
	SD	0·39	0·03
	n	31	10

Statistical analysis (Mann–Whitney *U*-test):

Mean epidermal thickness	Ulcer present vs. control	$w = 806$	$P < 0{\cdot}001$.
B/G ratio	Ulcer present vs. control	$w = 803$	$P < 0{\cdot}001$.

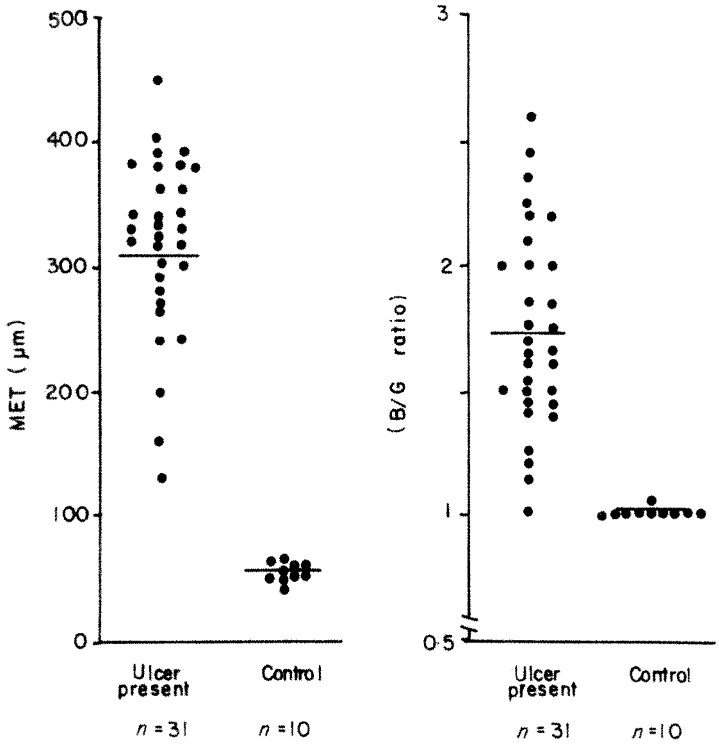

FIGURE 6. Measurements of mean epidermal thickness (MET-μm) and the length of basal layer : length of granular layer ratio (B/G ratio) in 31 patients with the gravitational syndrome and 10 normal healthy control subjects, assessed by computer assisted planimetry. The mean values are shown by horizontal bars.

TABLE 6. Correlation of vascular endothelial volume (VEV) and luminal volume (VLV) with age in patients and controls

Group	Measurement	Correlation coefficient	95% confidence interval on correlation coefficient
Patients	VEV	−0·005	−0·358 to +0·350
(n=31)	VLV	+0·049	−0·311 to +0·396
Controls	VEV	−0·017	−0·563 to +0·539
(n=10)	VLV	+0·066	−0·503 to +0·595

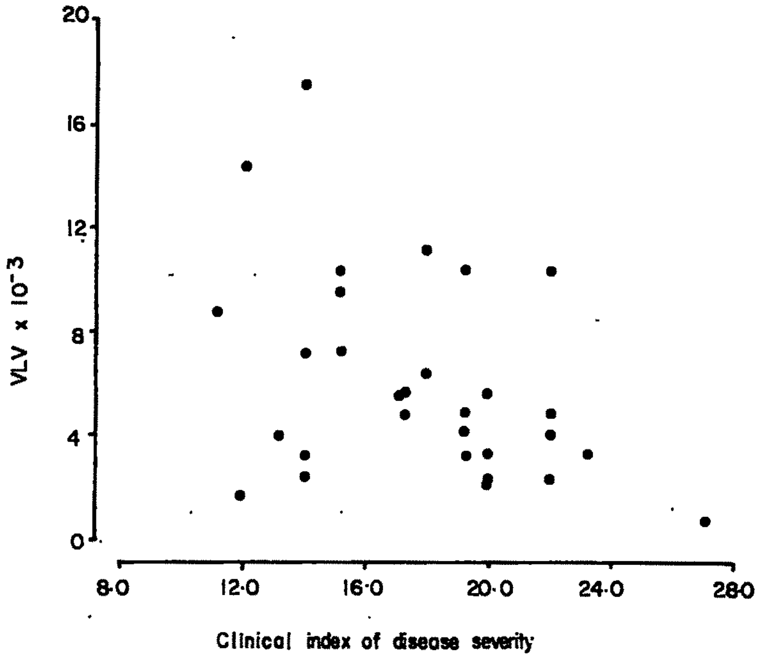

FIGURE 7. Scatter diagram to show the relationship between the clinical index of disease severity and vascular luminal volume (VLV) per unit tissue volume (× 10^{-3}) in gravitational syndrome patients. Spearman rank correlation, $R = 0{\cdot}365$, $n = 31$, $P < 0{\cdot}05$.

DISCUSSION

Angiogenesis is an essential part of the usual process of healing, yet there appears to be a profusion of new vessels in the non-healing wound in the gravitational syndrome. The explanation for this paradox is not clear. Information about the relationship between the endothelium of small dermal blood vessels and both clinical and other pathophysiological parameters of the disease would be helpful in understanding this syndrome. In this study vascular endothelial volume, luminal volume and the number of blood vessels in the dermis have

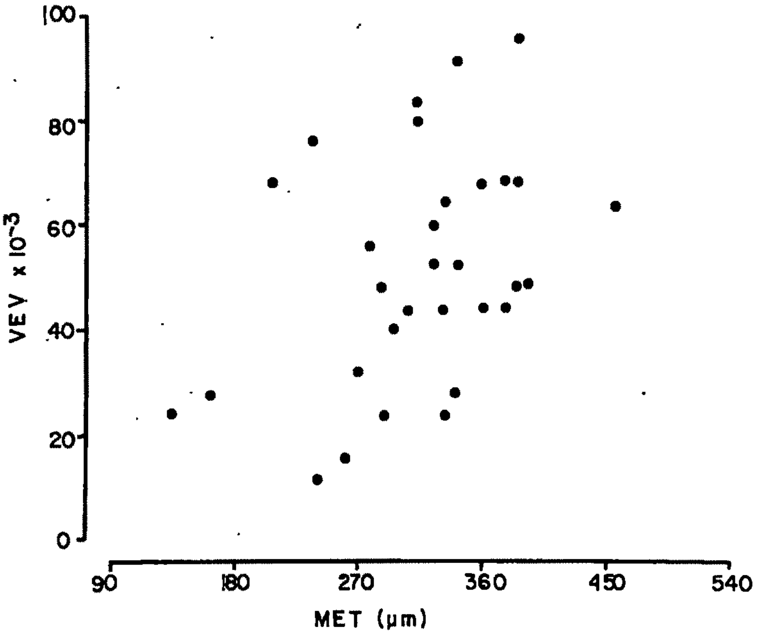

FIGURE 8. Scatter diagram to show the relationship between vascular endothelial volume per unit tissue volume (VEV) ($\times 10^{-3}$) and the mean epidermal thickness (MET) (μm) in gravitational syndrome patients. Spearman rank correlation, $R = 0{\cdot}361$, $n = 31$, $P < 0{\cdot}05$.

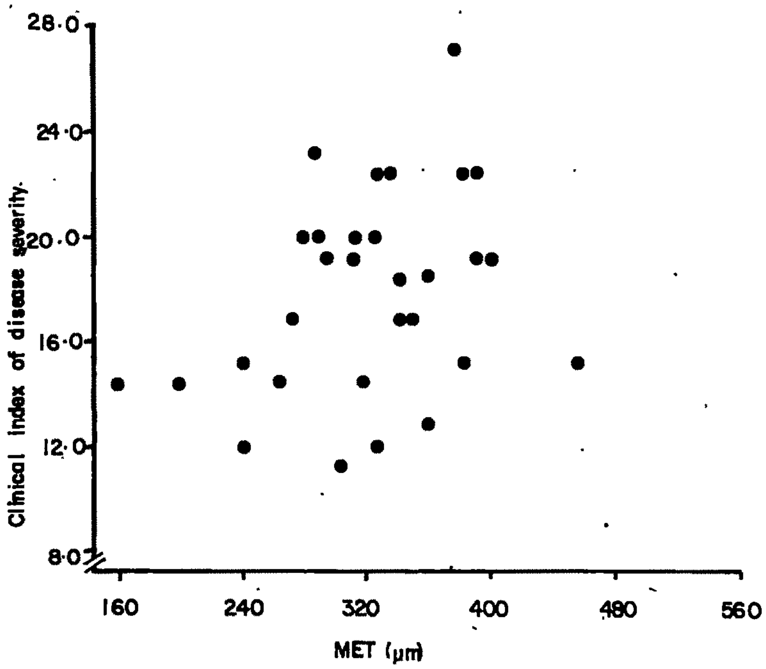

FIGURE 9. Scatter diagram to show the relationship between the clinical index of disease severity and the mean epidermal thickness (MET) (μm) in gravitational syndrome patients. Spearman rank correlation, $R = 0{\cdot}359$, $n = 31$, $P < 0{\cdot}05$.

been measured in patients with gravitational syndrome and compared with these in healthy control subjects. Semi-quantitative estimates of dermal vascular components have been made previously in the gravitational syndrome[1,2,9-11] but formal histometric analyses have not been reported prior to this study.

Difficulties were encountered in matching the age of the normal control subjects available (mean age 47 years) to the age of the patients (mean age 66 years). The range of ages in the control group, are included in the range of the patients' ages. There was no simple correlation with age of the parameters involved in this study and there was a marked difference between the values seen in the controls and the patients. These findings suggest that the results of this study are little influenced by the difference in age between the two groups.

The results for vascular endothelial volume, luminal volume and number of blood vessels per unit dermal tissue volume showed a significant difference between gravitational syndrome patients and control (Mann–Whitney *U*-test, $P<0{\cdot}001$, $P<0{\cdot}05$, $P<0{\cdot}01$ respectively). To ascertain whether the increased vascular endothelial volume and vascular luminal volume were simply the result of the increased number of blood vessels in the dermis, the ratio of vascular endothelial volume to the number of blood vessels (VEV:NV ratio) was calculated for each individual patient and normal control subject, and also the ratio of vascular luminal volume to the number of blood vessels in the dermis (VLV:NV ratio). Data expressed in this way did not reveal a statistically significant difference between gravitational syndrome patients and controls for both VEV:NV ratio and VLV:NV ratio. However, close examination of the control VEV:NV data (Fig. 3) shows one very high value—this specimen had the highest VEV (0·0343) and the lowest NV (0·86) in the control group. The remaining control values for VEV:NV ratio were all below the mean for the patient group. We therefore cannot completely discount the possibility that the increased VEV in gravitational syndrome skin is solely a function of greater endothelial mass.

The results for VLV:NV ratio showed that the increase in VLV in patients with the gravitational syndrome compared to that in controls was probably the result of an increased number of blood vessels and not the result of increased vascular luminal volume in the individual blood vessels. These results are in general agreement with those of Burnand *et al.*[2,11] in which semi-quantitative assessments of dermal vessel numbers and thickness were made. The wide inter-individual variation in each vascular parameter measured could be due to intrinsic tissue variability of the therapeutic regimens used during the healing process. In addition the parameters themselves may influence healing.

To clarify the relationships between the dermal vasculature and the clinical status the different parameters have been correlated with a clinical index of disease severity. A negative relationship was found between vascular luminal volume (VLV) per unit tissue volume and the clinical index of disease severity (Spearman rank correlation, $P<0{\cdot}05$). This suggests that vascular luminal volume per unit dermal tissue volume may help to indicate the prognosis of gravitational syndrome patients—the less the vascular luminal volume the more severely affected is the patient, leading to a poor prognosis.

Attention has been focused on the presence of perivascular fibrin deposition as a result of venous hypertension in the gravitational syndrome and the effect of this deposition on oxygen diffusion. The luminal volume, which obviously will influence volume flow in skin tissue, may also be of importance in ulcer healing but does not appear to have been adequately discussed as an influential factor on the healing process.

A positive relationship was found between vascular endothelial volume, luminal volume, the number of blood vessels per unit tissue volume, and the number of inflammatory cells in the dermal infiltrate (Spearman rank correlation, $P < 0{\cdot}05$, $P < 0{\cdot}01$, $P < 0{\cdot}05$ respectively). These results are supported by the reports of other investigators who have noted that various cell types have been found to induce growth of new capillaries. It was found that polymorphs and an extract from these cells induced corneal vascularization in experimental animals.[12] Hunt *et al.*[13] used wound inflammatory cells harvested by aspiration of fluid from the 'dead space' of subcutaneous rabbit wounds and transplanted them into the cornea and these were compared with suitable controls. Angiogenesis was stimulated and macrophages, as opposed to granulocytes, appeared to provide the major stimulus to angiogenesis. Macrophages that were activated by a non-specific inflammatory stimulus were more active than non-activated macrophages in inducing vascular proliferation in a similar model.[14]

A positive relationship has also been found between the vascular endothelial volume per unit tissue volume and the mean epidermal thickness (Spearman rank correlation, $P < 0{\cdot}05$). These results concurred with other data,[15-17] suggesting that vessels grow towards epithelium which may release an angiogenic factor, or it may be that epithelialization itself provokes new vessel formation. Other studies[18,19] have found marked epidermal hyperplasia at the margin of gravitational ulceration. The increased B/G ratio also suggested epidermal hyperplasia. The mean epidermal thickness was found to have a statistically positive significant relationship to the clinical index of disease severity (Spearman rank correlation, $P < 0{\cdot}05$). From the prognostic point of view the greater the epidermal thickness, the more severe the gravitational syndrome.

In conclusion, patients with the gravitational syndrome have increased numbers of blood vessels of increased vascular thickness but without increased luminal volume in their affected skin. By correlating these abnormalities with the clinical status of the patients it appears that these tissue parameters may assist in reaching a prognosis in individual patients.

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Types of human papillomavirus isolated from Japanese patients with epidermodysplasia verruciformis

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SUMMARY

Virological studies were performed on 12 patients with epidermodysplasia verruciformis (EV). Three types of lesions were observed: red plaques, pityriasis versicolor (PV)-like macules and plane warts. Human papillomavirus (HPV) 14, 20 and 21 were isolated from the plaques, HPV 3, 14 and 38 from flat warts and HPV 5, 12, 17, 20 and 38 from PV-like lesions. No clear relationship could be established between the different lesions and the types of HPV. Types 17 and 20 have been isolated most frequently from Japanese EV patients and HPV 5, frequently detected in other countries, is less common, whereas HPV 8 has not been isolated. Skin cancers occurred in six of the cases (50%) and all had benign lesions that were PV-like. At least one type of HPV 5, 17 or 20 could be isolated from these benign lesions and HPV 17 or 20 detected in the cancers. These three types of HPV in EV patients appear to be involved in the malignant transformation.

Epidermodysplasia verruciformis (EV) is an uncommon disease which usually begins in infancy or early childhood with various types of warts. Cutaneous carcinomas often develop later in life. Many types of HPV have been isolated from benign EV lesions and HPV5 and 8 have been detected in the cutaneous carcinomas of EV patients.[1] HPV 5 has also been shown to induce morphological transformation *in vitro*.[2] We have studied 12 patients with EV and we report the clinical and virological findings in these Japanese cases.

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METHODS

Patients

Twelve patients (nine male, three female) were included in this study. They were diagnosed as having EV on the basis of clinical and histological criteria.

Purification of HPV DNA

Tissue from EV lesions were snap frozen immediately after surgical removal and were homogenized and sonicated. The virus particles were purified with low-speed (5000 ***g***; 30 min) and high-speed (30 000 ***g*** ; 1 h) centrifugation and were then incubated at 37°C for 2 h in the presence of 0·5% sodium dodecyl sulphate and proteinase K (200 μg/ml). The DNA was extracted twice with phenol and once with choloroform:isoamyl alcohol (24:1) and precipitated with ethyl alcohol.

Determination of HPV types

HPV DNAs isolated from the patients were typed with Southern blot hybridization with 38 types (HPV 1–38) of standard HPV and then the cleavage patterns of isolated DNAs and standard HPV DNAs were compared. To confirm the HPV types, the isolated HPV DNA except HPV 5 and 12 were cloned and physical maps made.

Cloning

HPV DNA and plasmid pBR322 were digested by single cut endonuclease, ethanol precipitated and ligated with T4 ligase.The ligation mixture was used to transform *Escherichia coli* HB-101. Cloned HPV DNAs were digested by several endonucleases and the cleavage products separated with electrophoresis in agarose gel. The molecular weights of the fragments were evaluated by comparing the mobility of the fragments in ethidium bromide-stained gel with that of λ phage DNA fragments digested with Hind III.

RESULTS

Clinical findings

The cases are summarized in Table 1. Intrafamilial occurrence was seen in three families. Four of the patients were children of a consanguineous marriage and three were siblings. The average age of onset of the benign lesions was 12 years and 11 of the 12 patients had developed lesions before the age of 20. One patient who had developed EV lesions after the age of 20 had systemic lupus erythematosus and had been treated with corticosteroids and other immunosuppressive drugs for several years.

The benign EV lesions were classified into flat warts, red plaques and PV-like lesions. EV-specific flat warts resembling plane warts were seen but these were flatter and more red (Fig. 1). There were PV-like lesions that were small scaly macules (Fig. 2) and variously pigmented from a light to dark brown. A mixture of these coloured lesions occurred in the same patient. Flat warts were seen on the backs of the hands or arms of four of the patients and red plaques were mainly on the trunk with PV-like lesions over the whole body. Red plaques were seen in four of our patients and PV-like lesions in six with and in two patients without skin cancers. Biopsies of each type of lesion were taken and the histological findings were identical with swollen cells and pale-staining cytoplasm present in the upper spinous and granular layers of the epidermis associated with an increase in the amount of keratohyalin granules (Fig. 3).

TABLE 1. Summary of cases

Patient	Age	Sex	Age of onset	Type of eruption	Skin cancer	Intrafamilial onset	Consanguineous marriage
KM	36	M	7	PV, FW	SCC, Bo, BCE	+	+
JS	48	F	20	PV	SCC, Bo, BoCa	—	—
HT	32	M	11	PV	Bo	—	—
TN	48	M	7	PV	SCC	+	—
ST	44	M	6	PV	SCC	—	—
HT	54	F	7	PV	SCC, Bo, BoCa	—	—
NoF*	29	M	13	RP, PV, FW	—	+	+
HF*	28	F	11	PV, FW	—	+	+
NaF*	21	M	17	RP, FW	—	+	+
SS	20	M	6	RP	—	—	—
MH	26	M	23	RP	—	—	—
KH	34	M	12	PV	—	—	—

* NoF, HF, NaF: siblings.

PV: pityriasis versicolor-like lesion.
FW: flat wart-like lesion.
BCE: basal cell epithelioma.
BoCa: Bowen carcinoma.

RP: red plaque.
SCC: squamous cell carcinoma.
Bo: Bowen.

Six patients developed skin cancers. In all these cases the benign lesions were PV-like. Skin cancers were located on the sun-exposed areas in three patients and on the whole body in the other three patients. The average age of onset of skin cancers was about 40 years, and was much younger than for the general population. Various types of squamous cell carcinomas were seen and one patient had a basal cell carcinoma. Two patients died of malignant tumours, one from invasive and metastatic squamous cell carcinoma and the other from lung cancer.

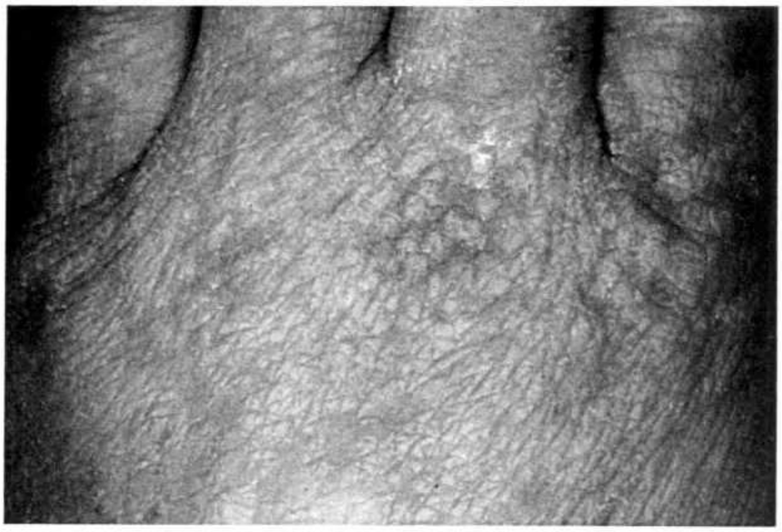

FIGURE 1. Flat wart-like lesions on the back of the hand of a patient.

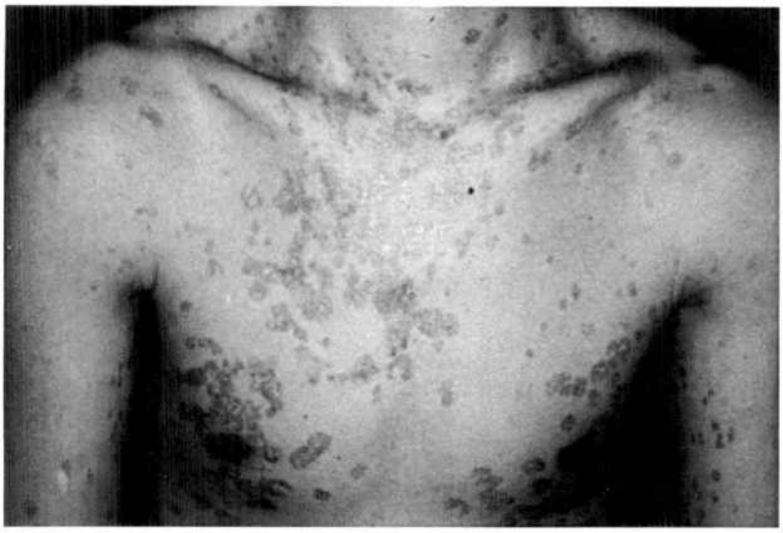

FIGURE 2. Pityriasis versicolor-like lesions on the chest.

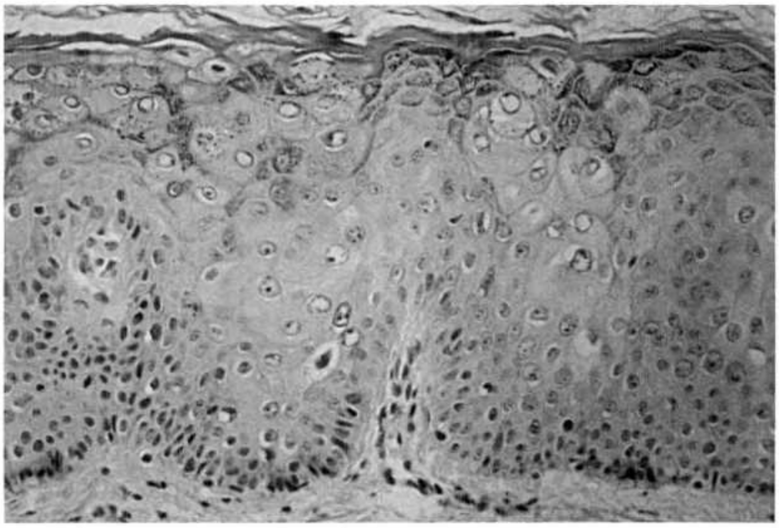

FIGURE 3. Skin biopsy showing swollen cells with pale-staining cytoplasm in the upper spinous and granular layers with an increase of keratohyalin granules (H & E, × 400).

Virological studies

The results are shown in Tables 2, 3 and 4. Nine types of HPV were isolated from the 12 patients: HPV 3, 5, 12, 14, 17, 20, 21 and 38 and one undetermined type. The undetermined HPV DNA was cross-hybridized with HPV 5 under stringent conditions but showed a different restriction pattern from that of HPV 5. HPV 3 was isolated from common or EV-non-specific flat warts, and eight types of HPV were isolated from EV-specific eruptions. HPV 17 was isolated from four patients (33%), HPV 20 from three patients (25%), HPV 5, 14 and 38 from

TABLE 2. HPV types isolated from pityriasis verisicolor-like lesions

Patient	Skin cancer	HPV types
KM	+	17, 20,* 38
JS	+	5
HT	+	17, 20
TN	+	12, 17
ST	+	17*
HT	+	5
HF	—	38
KH	—	ND†
NoF	—	14

* These two types of HPVs were also detected in cancer tissue.

† Not determined.

TABLE 3. HPV types isolated from red plaques

Patient	Skin cancer	HPV types
NoF	—	14
NaF	—	14
SS	—	21
MH	—	20

TABLE 4. HPV types isolated from flat wart-like lesions

Patient	Skin cancer	HPV types	Clear cells*
HF	—	38	+
NaF	—	14	+
NoF	—	3	—
KM	+	3	—

* Clear cells means swollen cells with pale-staining cytoplasm in the upper spinous and granular layers.

two patients (17%) and HPV 12 and 21 from one patient (8%). The incidence of isolation of HPV 17 and 20 was higher, but that of HPV 5 was lower, than that reported in other countries.

Seven types of HPV were isolated from the PV-like lesions of nine patients: HPV 17 from four patients, HPV 5, 20 and 38 from two patients each and HPV 12, 14 and one undetermined type from one patient. Three types of HPV were isolated from the red plaques of four patients: HPV

14 from two patients and HPV 20 and 21 from one patient each. Three types of HPV were isolated from flat wart-like lesions: HPV 3 from two patients and HPV 14 and 38 from one patient each.

Six patients developed skin carcinoma and HPV 5, 12, 17, 20 and 38 were isolated from their PV-like lesions—HPV 17 from four patients, HPV 5 and 20 from two and HPV 12 and 38 from one patient. HPV 17 and 20 were also detected in the cancer tissue of two of the patients. These five HPVs, particularly HPV 17 and 20, may be involved with carcinoma in EV patients.

DISCUSSION

In these cases, the incidence of intrafamilial onset was 30% and that of consanguineous marriage was 20%; other reports indicate a similarly high incidence.[3] Genetic factors are involved in EV. One patient who had systemic lupus erythematosus and was treated with corticosteroids and immunosuppressants developed EV. Immunosuppressed renal transplant patients have developed a variety of skin lesions induced by human papillomavirus,[4] and an immunological deficiency and susceptibility to HPV infection is likely in EV patients.

The red plaques were observed in relatively young patients and PV-like lesions in the older patients. HPV 20 was isolated from both types of lesions. Red plaques may represent the early stage of EV and this changes later on to the PV-like lesions.

Flat warts were mainly observed on the back of the hand and HPV 3, 14 and 38 were isolated from them. HPV 3 has also been isolated from verruca plana and HPV 38 from malignant melanoma,[5] but HPV 14 is EV-specific.[6,7] Plane warts and EV-specific flat warts appear similar, but the latter tend to be more flat and red.

HPV 5, 12 and 17 were isolated only from PV-like lesions and HPV 21 only from the red plaques, which may indicate a relationship between type of eruption and HPV types. However, HPV 14 was isolated from three types of eruptions, HPV 20 PV-like lesions and red plaques and HPV 38 from PV-like lesions and flat warts. In the case of patient NoF, whose eruption changed from red plaques to PV-like lesions, the same HPV (type 14) was isolated from both types of lesions. Thus, there is no clear relationship between types of clinical lesions and HPV types.

HPV 16 and 18 have been detected frequently in cases of cervical carcinoma in Germany,[8] but this type of HPV is less prevalent in Japan.[9] HPV 5 and 8 were isolated from many cases of EV in other countries,[7,10] while we found HPV 17 and 20 in several of our patients but no HPV 8 was detected.

Six of our patients developed skin cancer and in these the benign lesions were PV-like. At least one of HPV 5, 17 or 20 was isolated from their benign lesions and HPV 17 or 20 were isolated from the cancer tissue of two of the patients. This indicates that HPV 17 and 20 may have oncogenic potential, and it has been reported that HPV 5 is highly oncogenic.[1,11,12] The PV-like lesions may represent the later stage of EV, and malignant transformation may occur following a long period of infection with highly oncogenic viruses.

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Clinical and photobiological characteristics of xeroderma pigmentosum complementation group F: a review of cases from Japan

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SUMMARY

A 61-year-old female patient with xeroderma pigmentosum (XP), registered as XP46KO, was assigned to complementation group F by the cell fusion-complementation method. The XP46KO fibroblasts in culture exhibited a defective DNA repair capacity of 10–15% unscheduled DNA synthesis and a 3-fold sensitivity to the lethal effect of 254 nm ultraviolet light compared with normal cells. The patient had mild clinical symptoms consisting of numerous pigmented freckles and a small number of seborrheic keratosis-like papules. She had no skin cancers in the sun-exposed areas of the skin and so far no neurological abnormalities. A review of 11 Japanese group F patients revealed very mild skin symptoms with no ocular or neuro-psychiatric abnormalities. Single skin cancers occurred in only 3 of the 11 patients with an average age of 52 years for their first skin malignancy.

Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder with a high susceptibility to skin cancers due to defective repair of UV-induced damaged DNA.[1] XP currently comprises at least nine excision-defective complementation groups (A through I) and an excision-proficient XP variant group.[2–9] The distribution of XP patients among the various complementation groups is related to ethnic origin. World-wide, groups B, E, F, G, H and I are still rare.

The first female patient (XP23OS) assigned to group F in 1979[6] had very mild skin symptoms, despite a low level of unscheduled DNA synthesis (UDS) of 10% following 254 nm irradiation of fibroblasts. Further group F patients have been classified, and of these 10 are from Japan,[10] one from the U.K. (XP126LO)[11] and one from The Netherlands (XP42RO).[12] In this study we describe an additional group F patient (XP46KO) and report the clinical and cellular characteristics and a review of the other Japanese XP group F cases.

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PATIENTS AND METHODS

Patients

These group F cases were from the Kobe University XP registry.[13,15]

XP25KO. When aged 18 months she had severe sunburn, and when examined at the age of 8 years the sun-exposed areas of skin, especially the face, showed mild erythema with scaling and many pigmented freckles. Continuous photoprotection with a para-aminobenzoic acid (PABA) sunscreen has prevented further attacks of severe sunburn. There was no relevant family history of the condition.

XP27KO. (Fig. 1a) The patient's maternal grandparents were second cousins. Her mother had many freckles on the face, and she has two siblings (XP28KO and KP41KO) with similar photosensitivity of the skin. Erythema with oedema, scaling and pigmented freckles appeared in this patient after the age of three. She has no neurological abnormalities and her IQ at the age of 5 years was 93. The minimal erythema dose (MED) of UVB was within the normal range.

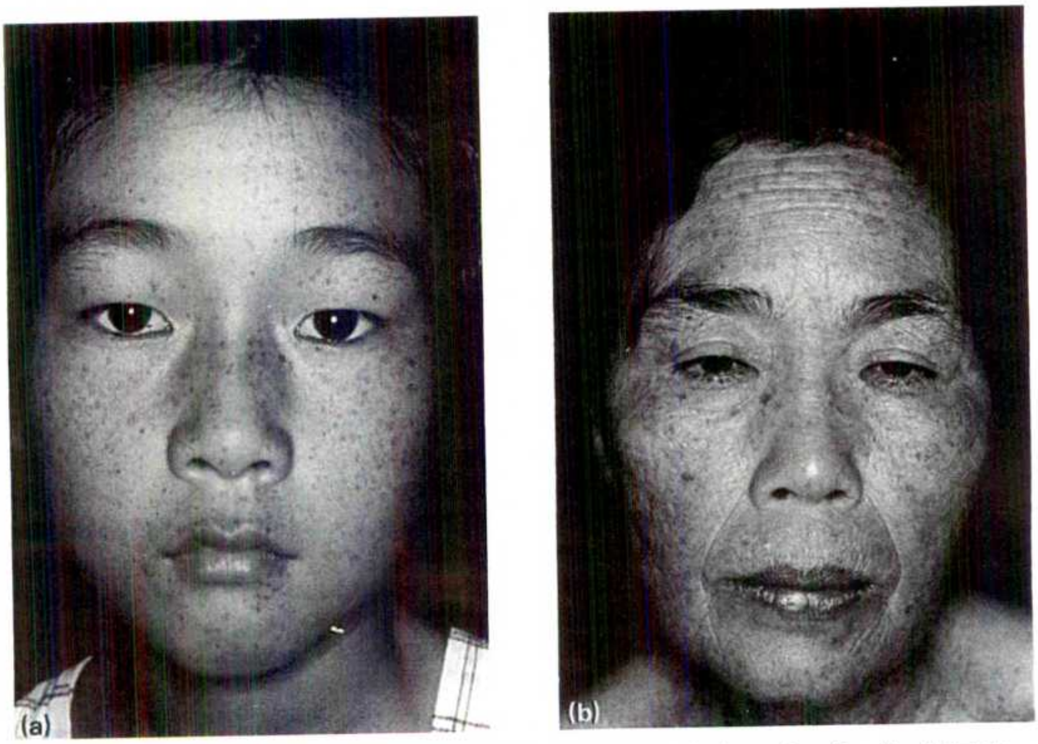

FIGURE 1. (a) Photograph of XP27KO, taken at 11 years old. She exhibited a number of small and slightly pigmented freckles a and small number of seborrheic keratosis-like verrucous papules on the cheeks and the bridge of the nose. However, neither apparent telangiectasia nor dryness of the facial skin was observed. (b) Photograph of 61-year-old female patient, XP46KO. Many freckles with slight to moderate brown pigmentation were noticed on the face, V-shaped area of the chest and the dorsa of the hands. She exhibited neither telangiectasis nor dryness of the skin, and developed neither malignant cutaneous tumours nor neurological abnormalities.

XP28KO. This patient is a younger sister of XP27KO, and had an acute episode of photosensitivity at the age of 3 years. Pigmented freckles began to increase in number at about 5 years of age. By the age of 8 she had seborrhoeic keratosis-like warty papules on her face and on the backs of both hands. Using a PABA sunscreen has not prevented an increase in the number of her freckles. No mental or neurological abnormality has so far been detected.

XP41KO. This patient is a younger brother of XP27KO. The telangiectasia first appeared on his face at the age of 7–8 months, and he had an episode of acute photosensitivity at the age of 3. By the age of 5 there were pigmented freckles on the sun-exposed areas of the skin. He has no mental or neurological abnormality.

XP38KO. Three siblings of this patient who were suspected of having XP were not examined. The patient had several episodes of acute photosensitivity during infancy and a severe episode of sunburn at the age of 7–8 years. Pigmented freckles have appeared on the face and their number is increasing. At the age of 44 years her skin symptoms were mild although there were numerous seborrhoeic keratosis-like warty papules on her face. She has no physical, mental or neurological abnormality.

XP46KO. (Fig. 1b) The XP group F assignment of this new case is shown in Figure 2. Her elder sister and two of her three sons had a similar photosensitivity, but were not examined. The patient is now aged 61 years and from the age of 4 has had recurrent severe attacks of sunburn with blistering of the skin following sun exposure. She developed freckles on the face at the age of 14–15 years. She was a farmer and, as a consequence, had much exposure to sunlight without adequate protection. The patient had not had any severe sunburn after the age of 20, and over the last few years the pigmentation on her face had become less apparent. There were pigmented freckles and seborrhoeic keratosis-like warty papules on the sun exposed areas. She has not developed any cutaneous malignancies and has no neurological problems.

Assignment of XP46KO to group F

The fibroblasts from the new patient, XP46KO, were derived from a biopsy taken from the flexor surface of the right upper arm. Cells were cultured in Eagle's minimum essential medium supplemented with non-essential amino-acids and 15% fetal bovine serum in an atmosphere of 5% CO_2 in air, as previously described.[14,15] The complementation of the fused heterodikaryons, identified by differential latex-bead labelling, was determined as previously described.[10,16] Briefly, XP46KO cells and reference cells—consisting of normal (N), NHSF6;[17] complementation group A, XP6KO;[14] group C, XP40KO;[15] group D, XP59TO[16] (gift of Dr Y. Satoh); group E, XP24KO;[18] group F, XP27KO;[13] and group G, XP2BI cells[7] (gift of Dr D. G. Harnden)—were separately pre-labelled for 24 h with large latex beads (LB, 1·09 μm in diameter; Dow Chemical Co.) and small beads (SB, 0·48 μm) respectively. After removal of extracellular beads, the co-cultures of LB- and SB-labelled cells (2·5 × 10^5 cells each/35 mm dish) were incubated overnight and fused with 45% (w/v) polyethylene glycol. The cells were replated sparsely on plastic coverslips 2 h later and incubated for 24 h to give sufficient complementation and then irradiated with 10 J/m^2 of 254 nm UV, and radioactively labelled with 10 Ci/ml of [methyl-^{3}H]-thymidine (53 Ci/mmol, Amersham) for 3 h in the absence of hydroxyurea. The coverslips were then processed for autoradiography using a Sakura NR-M2 nuclear emulsion. The mean number of grains per nucleus of dikaryon was estimated for a measure of UV-induced UDS by counting silver grains over 50 cells each of LB/SB-labelled heterodikaryons, LB-only labelled test-cell homodikaryons and SB-only labelled reference-cell homodikaryons. Thus, comparison of mean UDS in various heterodikaryons, relative to the normal-cell (NHSF6/NHSF6) homodikaryons, can distinguish whether or not genetic complementation occurs.

Cellular UV hypersensitivity

To determine clonogenic UV survival curves, exponentially growing cells were plated and allowed to attach for 4–5 h. They were then irradiated with graded doses of 254 nm UV, followed by incubation for 14 days of developing visible colonies of 50 cells or more. The 254 nm UV sensitivity of cells was determined in terms of extrapolation number (n) and mean lethal UV dose (Do) of the survival curve.[19]

Determination of minimal erythema dose (MED)

The MED of XP38KO and KP46KO patients was determined by exposing the dorsal skin of the upper trunk to 290 to 330 nm UV light using a Jasco Model CRM-FX monochromator.[14,20] The MEDs were measured 24, 48 and 72 h after irradiation, and the time of the maximum erythema reaction for each wavelength determined.

TABLE 1. Clinical phenotype characteristics of XP F patients in Japan

				Clinical phenotype				
				MED† at 300 nm	Severe sunburn	Cancers‡		
Patients	Age*	Sex	Parental relation	(mJ/cm^2)	(age at onset)	skin	other organs	Ref.
Kobe Univ Registry								
XP25KO	8	F	none	low (3·6)**	+(1·5)	—	—	13
XP27KO§	11	F	2nd cousin	normal	+(3)	—	—	13
XP28KO§	8	F		ND¶	+(3)	—	—	13
XP41KO§	5	M			+(3)	—	—	13
XP38KO	44	F	none	normal(7·3)	—	—	—	15
XP46KO	61	F	none	normal(14·8)	+(4~5)	—	—	present case
Cases from Other Institutions								
XP23OS	45	F		ND	+	—	—	6,21
XP101OS	49	F		ND	+	BCE (49)	—	25
XP2YO	65	F		ND		SCC (64)	bile duct cancer	22
XP3YO	29	M	none	ND		—	—	21,22,23
XP7KA	42	F	none	low (5·0)**	+(4~5)	BCE (42)	—	24

* Age at the first examination.

† Mean MED value (300 nm, 24 h) was 10·1 ± 3·0mJ/cm^2 for 43 normals. ** Indicates the MED values determined in other institutes.

‡ Skin cancers: BCE, basal cell epithelioma; SCC, squamous cell carcinoma. The numbers in the parentheses indicate the age for the first tumorigenesis.

§ Sibs.

¶ ND, not determined.

RESULTS

XP46KO: group F assignment, UDS and cellular UV sensitivity

The cases XP25KO, KP27KO, XP28KO, XP41KO and XP38KO in the Kobe University XP registry series had previously been allocated to complementation group F (see Table 1).[13] Figure 2 shows the result of the same complementation test for the new case, XP46KO. Setting the level of UDS of the NHSF6 normal-cell homodikaryons at 100%, only the heterodikaryons between the test cells XP46KO and reference group F XP27KO cells showed a non-complemented low level of 10–15% UDS after 10 J/m^2 UV, as did the XP27KO/XP27KO and XP46KO/XP46KO homodikaryons. All of the other heterodikaryons between XP46KO and the reference XP strains of group A, C, D, E and G showed nearly 100% UDS. Thus, we can allocate XP46KO to group F. The XP46KO homodikaryons exhibited the same level of UDS

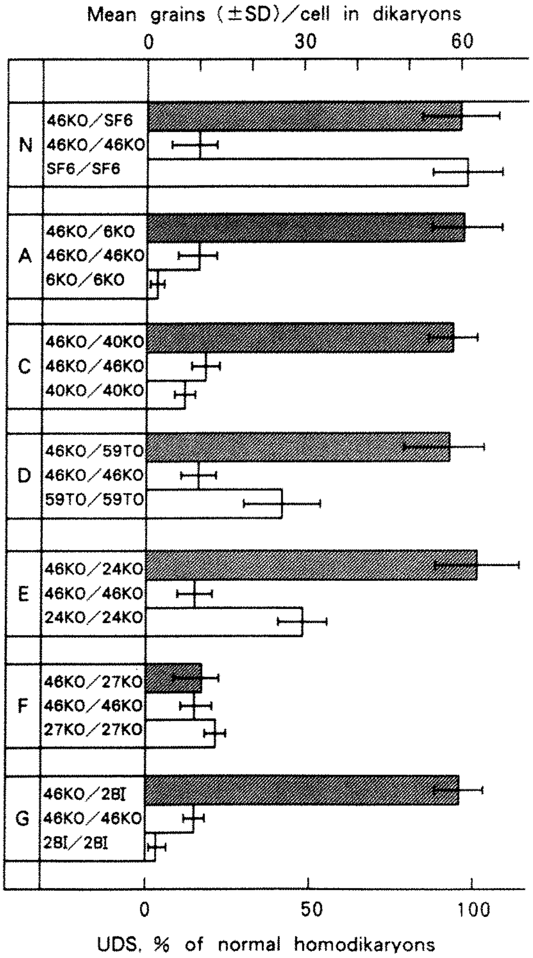

FIGURE 2. Complementation test for XP46KO. Relative unscheduled DNA synthesis (UDS) of hetero- and homodikaryons from different XP groups was expressed as a percentage of that of NHSF6/NHSF6 homodikaryons. Top horizontal axis shows mean grains per nucleus of dikaryons.

TABLE 2. Cellular hypersensitivity of XP group F

Patients	UDS* (% of normal)	Survival parameters†		Ref.
		n	Do (J/m^2)	
Kobe University registry				
XP25KO	10–15	1·2	1·7	13
XP27KO	10–15	1·2	1·7	13
XP28KO	10–15	1·2	1·7	13
XP38KO	20–25	1·7	2·2	15
XP41KO	ND‡	ND	ND	13
XP46KO	12	1·2	1·8	present case
Cases from other institutions				
XP23OS	10	1·0	1·7	6
XP101OS	10	1·0	1·8	25
XP2YO	10–15	1·2	1·7	22
XP3YO	10–15	1·2	1·7	21,22,23
XP7KA	19	ND	ND	24

*Determined autoradiographically after incubation for 3 h with [^{3}H]thymidine following 10J/m^2 254 nm UV.

†5 normal cell strains have $n = 1·5 \pm 0·02$ and Do $= 5·0 \pm 0·2$J/m^2.

‡ND, not determined.

(10–15%) as the previously assigned group F strains, apart from XP38KO (20–25% UDS) (Table 2).[15] The complementation test with group B (XP11BE), H (GM3248) and I (XP3MA) was not carried out, because no XP strain has been shown to share more than one complementation group.

The analysis of the UV survival curve revealed that based on the Do comparison, the XP46KO fibroblasts were about three times more sensitive to 254 nm UV killing ($n = 1·2$, Do $= 1·8$ J/m^2) than normal cells ($n = 1·5$, Do $= 5·0$ J/m^2) (Fig. 3), as were the other group F strains (Table 2).

MED

The results of monochromatic UVB- and UVA-induced erythema in 45 normal subjects (aged 1–67 years) compared with the group F patients XP38KO and XP46KO are shown in Figure 4. Neither patient showed any delay in the maximum erythema reaction and both had a normal reaction to UV radiation. The 24 h MED curves of both XP patients were within the normal range, although the 290 nm MED value ($= 5·3$ mJ/cm^2) for both XP46KO and XP38KO seemed slightly lower than the lower limit of the normal control.

DISCUSSION

The clinical features of the 11 Japanese group F patients—six from the Kobe University XP registry series (XP25KO, XP27KO, XP28KO, XP41KO, XP38KO, XP46KO) and five from

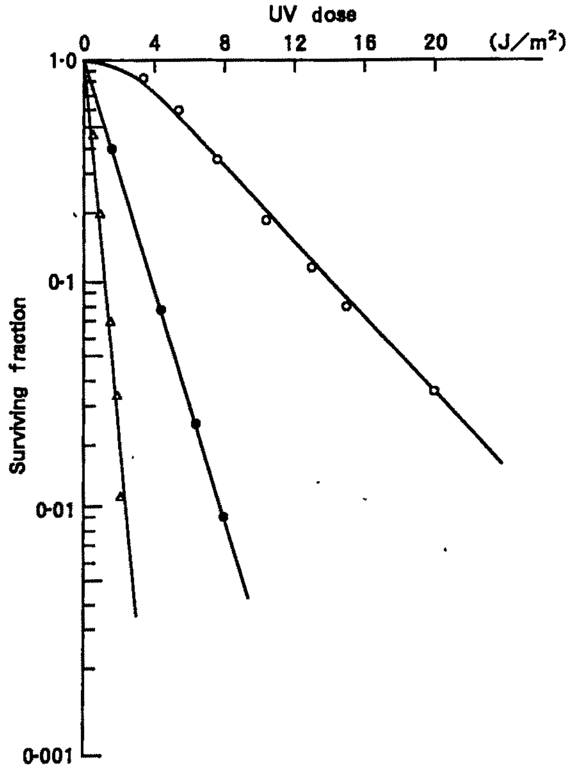

FIGURE 3. Survival curves of fibroblasts exposed to 254 nm UV. Open circles indicate NHSF6, normal cell strain (Do = 5·0 J/m², n = 1·5); closed circles, XP46KO, group F (Do = 1·7 J/m², n = 1·8); open triangles, XP35KO, group A (Do = 0·5 J/m², n = 1·0).

the other institutions (XP23OS,[21] XP2YO,[22] XP3YO,[23] XP7KA,[24] XP101OS[25]) are reviewed and are shown in Table 1. The clinical characteristics are a delayed occurrence of sunburn in childhood from 1·5 to 5 years as compared with the more severe and earlier sunburn in infancy (usually 3–6 months) in most XP group A children; the delayed development of light-brown freckles at the age of 5 years, apart from the freckles in XP46KO that appeared at about 14 years of age; the mild skin signs as characterized by freckles and small numbers of seborrhoeic keratosis-like pigmented papules with no apparent xerosis. In these patients there were no ocular or neurological abnormalities even in those in the older age groups. There was a low incidence of skin tumours even in the adults. Thus group F patients, like group E, have only mild skin symptoms and no neurological abnormalities. Distinguishing this disorder from other skin conditions with multiple pigmented freckles is difficult unless DNA repair analysis and the complementation group tests are performed.

Photosensitivity in XP patients usually occurs as severe sunburn with oedema and blistering in infancy and childhood,[3,14,26] and the extent of cutaneous photosensitivity of XP patients to UVB and UVA can be determined. Martenstein first described the delay in the development of the erythematous reaction and the marked hypersensitivity of XP subjects to UV radiation.[27] Sensitivity to the UV range of 280–310 nm was found,[28] with a delayed peak reaction of erythema at 297 nm at 48–72 h.[29] The action spectrum of MED after monochromatic UV

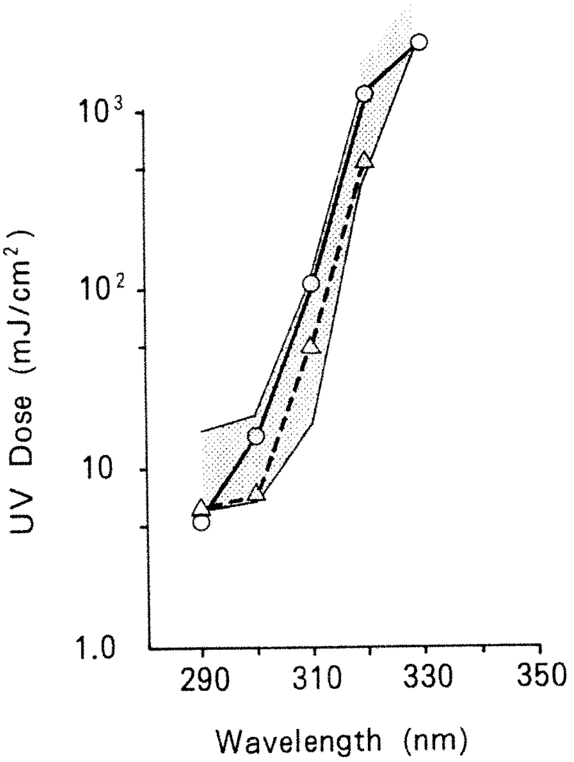

FIGURE 4. Minimal erythema dose (MED) curves for UVB-induced erythema observed at 24 h after irradiation. MED of XP38KO (○), XP46KO (△) and range of normal subjects (shaded area).

irradiation of an unassigned XP patient with neurological abnormalities was compared with those of 19 controls, and the maximum erythema at 72 h and a longer persistence of erythema was confirmed.[30] Both of our group F patients tested exhibited the maximum erythema reaction 24 h after irradiation and had an almost normal MED, although both had a slightly lowered MED at 290 nm. This reaction was quite different from the abnormal erythema pattern in group A and D patients,[14,30-32] who have a delayed peak reaction and low MEDs especially at wavelengths 290–310 nm. However, the MED at 300 nm of a younger XP patient (XP25KO, 8 years of age), measured by K.N. using a similar monochromator in a different institution, was slightly lower, although the peak erythema reaction was not delayed. An XP group F case (42-year-old female, XP7KA) was reported as showing a delayed erythema reaction at 72 h and a slightly lower MED at 24 and 72 h after 300 nm UVB radiation.[24] Thus, there is variation in the erythema reaction within group F, as occurs in the less photosensitive complementation group E.[18,33]

Group F cells usually exhibit a low level of 10–15% UDS for 3 h post-UV, with a higher residual level of 50% cumulative UDS during a period of 16–24 h after irradiation.[15,34] Group F cells showed three times more hypersensitivity to 254 nm UV killing than normal cells, which is due to the slow repair (UDS) of DNA. The higher level of repair in XP38KO cells (25% relative UDS) shows that there is heterogeneity within group F, which has also been found in groups A,[35,36] D[37] and G.[20]

About a quarter of the group F patients (three out of 11) in Japan had a single cutaneous malignancy (one squamous cell carcinoma and two basal cell epithelomas). These occurred at the relatively older age of between 42 and 64 years (the average age for the appearance of the first skin cancer was 51·7). One (XP2YO)[22] had a bile duct cancer at the age of 65. However, the 44-year-old XP38KO and the 61-year-old XP46KO who were both farmers and frequently exposed without adequate photoprotection to sunlight have, as yet, no skin malignancies. A recent survey of skin carcinogenesis in Japanese XP patients[10] has indicated an incidence of skin cancer of approximately 40% with the average age for the first appearance of a tumour of about 10 years in those assigned to group A and with less than 5% UDS. Group F patients however, show a delay in the development of skin cancers that is probably due to the restoration of 60% relative UDS at 20–24 h after irradiation.

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Transmission of virus particles by cryotherapy and multi-use caustic pencils: a problem to dermatologists?

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SUMMARY

The possibility of transmission of virus particles from patient to patient by multi-use silver nitrate sticks and styptic pencils and by cotton wool swabs that have been dipped repeatedly into Dewar flasks of liquid nitrogen was studied. The results showed that transmission by the sticks and pencils is unlikely but that virus particles can be transferred into and out of a Dewar flask on cotton wool swabs. We suggest that swabs should not be dipped repeatedly into the flask of liquid nitrogen but that, instead, a small aliquot of nitrogen should be decanted into a smaller 'clean' vessel and a new cotton swab used for each patient.

Liquid nitrogen (LN) is used extensively by dermatologists to treat a wide range of lesions. Ideally, this is delivered with a no-touch technique using an instrument such as the Cryowen®, but these and similar instruments are costly. Frequently, therefore, the cheaper alternative of a Dewar flask and cotton wool swabs are used, especially for viral warts. This involves the repeated dipping of the swab into the LN, turning it into a 'multi-use' modality. Other commonly used 'multi-use' treatments include silver nitrate sticks (SNS) and aluminium sulphate (styptic) pencils (SP), often used for cautery or haemostasis.

Transmission of virus from doctors to patients through surgical procedures[1] and the possible dispersal of virus by Hyfrecator® electrodes[2] have already been reported. The possibility that the above 'multi-use' treatments could also result in virus transmission from patient to patient has been suggested.[3] One study has shown that virus may survive on a cotton swab dipped into LN[4] and suggests that virus transmission from patient to patient may occur via this route. The present study was designed to investigate this possibility further.

The viruses which may possibly be transmitted by these routes include: (i) human papillomavirus (HPV) when treating warts; (ii) hepatitis B (HBV) and human immunodeficiency (HIV) viruses if bleeding or weeping lesions are treated; (iii) herpes simplex virus (HSV).

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Since HPV and HBV do not grow in routine cell cultures and HBV and HIV are hazardous to handle, other viruses were used as models in this work. HSV was used as a virus of significance in its own right and to represent the relatively labile HIV. An adenovirus (non-enveloped) was used to represent the hardier HPV and HBV. In addition, specimens were examined for HPV by electron microscopy (EM).

METHODS

Viruses

Herpes simplex virus type 1 (HSV 1) strain SC 16[5] was obtained from the Bristol Public Health Laboratory (PHL). Stocks were prepared and titrated by plaque assay in Vero cells grown in Medium 199 (Flow Laboratories, Irvine, Scotland, U.K.) supplemented with 6% fetal calf serum (FCS) for growth medium (GM) and 2% FCS for maintenance medium (MM).

Adenovirus type 2 (AD 2) was obtained from the Bristol PHL. Stocks were prepared, typed and titrated in Hep-2 cells grown in Eagle's medium (Flow Laboratories) supplemented with 10% FCS (GM) and 2% FCS (MM). For EM studies, the clarified cell culture preparations were concentrated by ultracentrifugation.

Infectivity assays

The infectivity of HSV was measured by plaque assay[5] under a methyl cellulose overlay containing MM in 24 well cell culture trays (Flow Laboratories). Titres are expressed as plaque-forming units (PFU) per ml. The infectivity of the non-plaque-forming adenovirus was measured by an end-point assay[6] in Hep-2 cells in either 24 (Flow Laboratories) or 96 well (Sterilin Ltd, Feltham, Middx, U.K.) cell culture trays. A 50% end point (EP_{50}) of cytopathic effect was estimated and titres expressed as EP_{50} per ml.

Electron microscopy

The presence of virus particles and viral morphology were assessed by the examination of inoculated negatively-stained (1·5% phospho-tungstic acid pH 6·5) grids by electron microscopy (Philips CM 420).

Chemicals

When silver nitrate ($AgNO_3$) and aluminium sulphate $Al_2(SO_4)_3$ pencils are used in therapy, near-saturated concentrations are likely to be reached. In these experiments, therefore, such conditions were reproduced by dissolving silver nitrate caustic pencils (Avoca Pharmaceuticals Ltd) or aluminium sulphate styptic pencils (Avoca) in small volumes of sterile de-ionised water. Liquid nitrogen was obtained from British Oxygen Ltd.

Experimental design

Effect of silver nitrate and aluminium sulphate on virus morphology. A drop of concentrated virus suspension was mixed with an equal volume of $AgNO_3$ or $Al_2(SO_4)_3$ solution. This was then applied to formvar coated grids (made of nickel, since copper reacts with $AgNO_3$) which were then washed and stained.

Effect of silver nitrate and aluminium sulphate on virus infectivity. Saturated solutions, 0·5 ml of $AgNO_3$ or $Al_2(SO_4)_3$ were added to 1 ml of stock preparations of herpes and adenovirus. The resulting mixtures immediately turned cloudy. After varying periods of time ranging from 0 to

60 min, the solution was centrifuged at 2000 *g* for 10 min and the supernatant removed and diluted with 20 ml of MM to dilute out the $Al_2(SO_4)_3$ or $AgNO_3$. Further precipitate formed and was removed by spinning at 4000 *g* at 4°C for 10 min. The resulting suspension was then spun in a high speed centrifuge at 19000 *g* at 4°C for 2 h to pellet the virus particles. Control samples of virus solution mixed with de-ionized water were put through the same protocol. After spinning, the supernatant was removed and the virus pellet was resuspended in the relevant MM and inoculated onto Vero or Hep-2 cells in culture trays.

Survival of virus in Dewar flasks after LN exposure. Aliquots (1 ml) of stock virus suspension (diluted 1 : 1 with fetal calf serum to simulate association with organic matter *in vivo*) were added to Dewar flasks of LN and the LN allowed to evaporate. After thawing, aliquots were taken from the residual solution and assayed for infective virus particles. The flasks were then allowed to dry overnight and the bottom of the flask was then washed with MM and an aliquot assayed for infective virus, the process being repeated at 24-h intervals.

Ability of a swab to introduce virus from a Dewar flask. The tip of a cotton wool bud which had been 'charged' with LN was dipped into a solution containing either herpes or adenovirus, the swab being removed before complete thawing as is routine in clinical practice. The swab was then 'recharged' in the LN flask and the process repeated three times. After all the LN in the 'recharging' flask had evaporated, it was washed with MM and this was assayed for infective virus particles.

Ability of a swab to retrieve virus from a Dewar flask. One millilitre of stock virus solution was added in small drops to a Dewar flask of LN. Cotton wool swabs were then dipped into the flask at hourly intervals. The swabs were then washed in maintenance medium which was assayed for infective virus.

Presence of virus in wart parings and swabs used to treat warts. The presence of virus in parings from warts along with the cotton wool swabs subsequently used to treat the same warts was determined by EM, the samples being stored at −70°C.

Presence of virus in Dewar flasks after routine use. Dewar flasks which had been routinely used in the clinic without particular care being taken over swab renewal were washed with MM after the residual LN had evaporated. The MM was then assayed for virus by culture (on Vero and Hep-2 cells) and by EM.

RESULTS

Effect of silver nitrate and aluminium sulphate on virus morphology

The control grids showed typical virus morphology as did grids from suspensions treated with $Al_2(SO_4)_3$. No particles, however, were seen after $AgNO_3$ treatment. Presumably, the $AgNO_3$ destroyed virus morphology, although we cannot rule out the possibility that the chemical prevented the adhesion of virions to the formvar on the grid.

Effect of silver nitrate and aluminium sulphate on virus infectivity

Infective virus was not detected in any of the experiments performed, whatever the exposure time of virus to $AgNO_3$ or $Al_2(SO_4)_3$. Infective virus was always isolated, however, in the control experiments. Although the shortest incubation was intended to be only for a few

TABLE 1. Survival of virus in Dewar flasks after LN exposure

	Original	Boiled Off	24 h	48 h	72 h
Adenovirus (EP_{50}/ml)	10^6	10^6	10^6	0	0
Herpes (PFU/ml)	21×10^6	21×10^6	19×10^4	0	0

seconds, in practice it was probably nearer 15 min before the concentration was reduced by dilution. The repeated washes carried out were found to be essential to prevent the $AgNO_3$ or $Al_2(SO_4)_3$ killing the cells on the culture trays.

Survival of virus in Dewar flasks after LN exposure

There was no drop in the titre of either herpes or adenovirus immediately after thawing. After 24 h, both viruses could still be isolated from the flask although with approximately a 100-fold loss of titre in the case of herpes virus. After 48 h, however, no infective particles of either virus were found (Table 1).

In a further experiment, adenovirus suspensions were added directly to replicate wells in cell culture trays, different wells being inoculated daily for 6 days. The trays were left at room temperature and on the sixth day, Hep-2 cells were added to all wells (which had therefore contained virus for between 1 and 6 days) and the cultures subsequently examined for cytopathic effect. This further experiment was included as it was suspected that repeated dehydration and rehydration may have reduced the viability of the virus particles in the initial experiment, reducing the survival period of virus in the dry flask.

The results confirm that under these conditions, adenovirus can remain viable for at least 6 days. The same experiment was not carried out for the less hardy HSV which would not be expected to remain viable over these periods even without repeated dehydration and rehydration.

Ability of a swab to introduce virus into a Dewar flask

The total amount of herpes virus in the flask after the LN had evaporated was $1{\cdot}4 \times 10^3$ (PFU/ml) as compared with $9{\cdot}5 \times 10^4$ (PFU/ml) in the stock solutions, implying a carry over of about 15 μl of original virus solution. Adenovirus was not specifically titred, but infectious virus was found in each of the 20 samples into which the 1 ml washing was divided. These experiments show, therefore, that a swab could transfer virus particles into a flask of LN into which it was dipped, although, not surprisingly, the amount of virus transferred was small.

Ability of a swab to retrieve virus from a Dewar flask

Whilst a flask was full, infectious virus was not picked up by a swab dipped into it. However, once the LN had evaporated, infective virus was retrievable from the bottom of the flask with no loss of titre.

Presence of virus in wart parings and swabs used to treat warts

HPV particles were frequently seen by EM in parings taken from viral warts (mostly verrucas).

In one of two cases investigated, virus particles were also detected on the swabs used to treat these warts, suggesting that this could be a mode of virus transfer in the clinical setting.

Presence of virus in Dewar flasks after routine use

On no occasion was infectious virus isolated from a flask after routine use. By EM, however, papillomavirus particles were seen in small numbers on two of three occasions.

DISCUSSION

With regard to silver nitrate and aluminium sulphate sticks, the above results are reassuring. Although virus particles seem to survive exposure to $Al_2(SO_4)_3$ and were morphologically intact, no infective virus particles were found after exposure to either chemical. The nature of the experiments meant that the minimum exposure time was probably in the order of 15 min, a period likely to be shorter than the time between patients in a clinical setting. Even at this time, however, no infective virus particles were found. In clinical practice the concentration of the solution on the tip of a stick is likely to be even higher than that used in our experiments. We suggest that virus transmission by this route is highly unlikely, even if the time period between the repeated use of the stick is short.

With regard to LN, contrary to the widely held view among clinicians, LN does not kill virus, and is routinely used to store virus in the laboratory. Our experiments show that not only do herpes and adenoviruses survive freezing and thawing, but the latter at least can remain viable in the bottom of a Dewar flask for several days. We have also shown that it is possible (in the laboratory) to transfer virus into and out of a Dewar flask by means of a cotton wool swab, although transmission out only took place after the LN had evaporated. In addition, our clinical studies have demonstrated that in routine medical practice, virus particles can be picked up from a verruca on a cotton swab and could potentially infect a flask into which the swab is dipped. Indeed, papillomavirus particles were demonstrable in LN flasks after routine clinical use. Although no virus was cultured from the flasks, this is not particularly reassuring as, of the viruses listed in the introduction, our culture system would only detect HSV which we would have been unlikely to have been found in the clinical settings studied.

We suggest, therefore, that the multi-use sticks such as $AgNO_3$ and $Al_2(SO_4)_3$ are likely to be safe from a virological point of view. In contrast, however, it seems quite possible that virus particles could be transferred from patient to patient by repeated dipping of a cotton wool swab into a flask of LN. This suggestion is supported by a report which described two patients who developed warts after cryosurgical treatment of non-viral lesions with cotton tipped applicators previously used to treat warts on other patients.[7] We propose that a new swab is used for each patient and that swabs are not dipped repeatedly into the flask. Ideally, the LN should be aliquoted into a disposable vessel (such as a polystyrene cup) or a small dish which is cleaned between patients. If swabs are dipped into the main Dewar flask, or if the smaller container is not changed or cleaned between patients, then they should be kept well filled. The reasons for this are that in our experiments transfer did not occur from a full container, and that, although we only recovered virus from a totally thawed flask, tissue debris (which accumulates at the bottom of a container) could, in theory, be picked up by a swab dipped into a virtually empty flask. As an additional precaution, unused LN in the small vessel should not be returned to the Dewar flask, the contents of which should not be returned to the main LN store at the end of the day. Finally, the Dewar flasks should be washed after each clinic session with gluteraldehyde or sodium hypochlorite (Chloros) for 15–30 min. In practice this is most easily done prior to refilling, the LN having evaporated overnight.

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Thyroid disease in pustulosis palmoplantaris

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SUMMARY

An increased frequency of thyroid autoantibodies has been reported in patients with palmar and plantar pustulosis (PPP). This study was undertaken to determine the frequency and type of thyroid disease in 32 patients with this disease compared with a control group. Thyroid disease was demonstrated in 53% of the patients with PPP as compared to 16% in the matched control group. Fourteen patients with PPP had an enlarged thyroid and in six there were thyroid autoantibodies. There appears to be an increased incidence of goitre and thyroid autoantibodies in PPP with a decrease in the level of the thyroid hormones.

The chronic course of pustulosis palmoplantaris (PPP) is well known,[1,2] and abnormalities in the normal immunological responses and correlation with infectious diseases have been claimed.[3,4] Also, a relationship with rheumatic disease has been suspected.[5] However, the aetiology of the disease remains obscure. An increased prevalence of thyroid autoantibodies in patients with PPP has been reported,[6] and screening tests for thyroid disease in patients with PPP have been recently advocated.[7] The aim of the present study was to determine the frequency and type of thyroid disease in patients with PPP as compared with a matched control group.

PATIENTS AND METHODS

Over a 4-year period, 44 patients with PPP attended our clinic. Twelve were excluded from the study for the following reasons: three had moved to another area of the country, four did not want to participate because of medical or mental diseases, and five patients because of old age and the inconvenience of attending the clinic for the tests.

Of the 32 patients included in the study 25 were female and seven male. The mean age was 55·5 years (± 14·3). Thirty-two randomly selected subjects from a population study, matched with regard to sex, age, body weight and smoking habits served as controls.[8] Informed consent from the patients was obtained, and the study was approved by the local ethical committee.

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A full history was taken from all the patients, including whether they had any skeletal symptoms[9] and a full clinical examination was carried out. In this study thyroid disease was defined as being: (i) a clinically detectable goitre; (ii) treatment of thyroid dysfunction; (iii) a previous thyroidectomy; (iv) the presence of thyroid autoantibodies in serum. The presence of a goitre was defined as a visible and/or palpable thyroid gland. Ultrasonic scanning was performed and the total volume of the thyroid was calculated as previously described.[8] Transverse scans of the thyroid were obtained in the supine position at 5 mm intervals from caudal end to cranial end with hyperextension of the neck, and the outline and the volume of the thyroid gland determined.[10]

The serum thyrotropin (TSH), thyroxine (T_4), triiodothyronine (T_3) and triiodothyronine resin uptake (T_3RU) were determined. Free T_4 index and free T_3 index levels were calculated as total hormone concentrations × T_3RU and given in arbitrary units. The thyroglobulin antibodies (TgAb) were measured[11] and microsomal antibodies (MsAb) were detected using an immunofluorescence technique.[12] Autoantibodies against salivary gland, adrenal cortex, gastric parietal cells, smooth muscle, mitochondria, extractable nuclear antigen, antinuclear, anti-DNA, IgM rheumatoid factor, granulocyte-specific antinuclear were measured using standard methods.[13-16]

Statistics

The Student's t-test was used when comparing mean values between groups. A significance level of 5% was chosen. Fisher's exact test was used when comparing frequencies.

RESULTS

Twenty-eight of the patients at the time of the study had active lesions of PPP involving the palms and soles. Four had no signs, but the diagnosis was confirmed from their previous medical records. The mean age at onset of the disease was 47·5 years ± 13·6. None had psoriasis. The mean duration of the illness was 8·0 years (± 9·9 years) and none had the condition for less than 6 months. Seven of the patients had skeletal symptoms; three complained of pain in the

TABLE I.

	PPP	Controls	P-value
Age (years)	56 (14)	56 (14)	NS
Bodyweight (kg)	68 (12)	70 (8)	NS
T_4	92 (17)	99 (17)	NS
T_3	2·22 (0·48)	2·21 (0·34)	NS
T_3-test	0·88 (0·14)	1·01 (0·12)	$P<0·001$
TSH	1·92 (1·04)	2·10 (1·22)	NS
FT_4I	79·2 (13·2)	99·7 (16·5)	$P<0·001$
FT_3I	1·90 (0·31)	2·22 (0·34)	$P<0·001$
Thyroid volume	27·1 (19·5)	22·6 (8·7)	NS

Median values and standard deviations of age, bodyweight, T_4, T_3, T_3-test, TSH, FT_4I, FT_3I and thyroid volume for patients with pustulosis palmoplantaris (PPP) and a matched control group (controls). Significance level was $P<0·05$. NS = not significant.

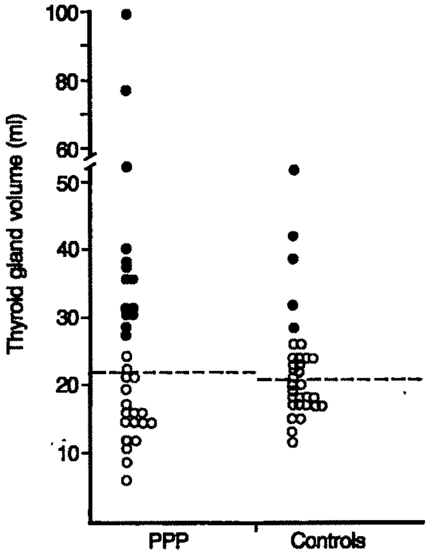

FIGURE 1. Thyroid gland volume in patients with pustulosis palmoplantaris (PPP) and matched controls. Subjects with (●) and without (○) clinically detectable goitre. Mean thyroid gland volume is indicated by the broken horizontal lines.

sternoclavicular joint, two of pain in shoulder and knee joints and two had been diagnosed as having rheumatoid arthritis.

Seventeen of the patients (53%) had one or more signs of thyroid disease. Fourteen patients had goitre, although two had received treatment with carbamazepine, which is known to cause a goitre.[17] Six patients had serum thyroid autoantibodies (six had MsAb and two also had TgAb). Two patients had a previous thyroidectomy, and one had radioiodine for hyperthyroidism which is known to decrease thyroid size.[18] In the control group five subjects (16%) had one or more signs of thyroid disease: five had a goitre and two had serum thyroid autoantibodies. All these subjects smoked cigarettes which is a known cause for a goitre.[19] A significant difference in the frequency of thyroid disease was demonstrated between PPP (17/32, 53%) and controls (5/32, 16%) ($P < 0{\cdot}005$).

The clinical data and thyroid function tests are given in Table 1 and Figure 1. The serum FT_4I and FT_3I levels were significantly lower in the PPP group than in the controls, but no significant difference in the value of serum TSH was found. The thyroid volume did not differ significantly between the groups (Fig. 1).

DISCUSSION

This study indicates an increased incidence of thyroid disease in PPP. One or more signs of thyroid disease were demonstrated in 17 of our 32 patients (53%) as compared with five of 32 controls (16%). Sex ratio, mean age at onset of PPP and mean duration of PPP did not differ from earlier studies.[1] The number of patients with skeletal complaints was relatively smaller than that reported in a recent paper,[9] but in our study only the subjective symptoms were recorded and no other investigations were carried out.

The most frequent sign of thyroid disease was a goitre, and this occurred in approximately half of the patients. Numerous factors influence thyroid size, e.g. sex, age and bodyweight,[8] seasonal variations,[20] time of menstrual cycle,[10] smoking habits,[19] the use of several drugs and numerous other factors. It is necessary that the control group is matched with regard to these variables. The relatively high frequency of goitre of 5/32 (16%) in the control group could be explained by their smoking habits,[19] as all were heavy smokers. No significant difference in mean thyroid volume could be demonstrated between patients and controls. However, of the patients, two had a thyroidectomy and one was treated with radioiodine. The inclusion of the pretreatment size of thyroid gland in these patients would have increased the difference in the frequency of goitre in our study.

An increase in serum TSH level could have explained the increase in frequency of goitre and the failure to find such a difference is not related to methods used. The fact that FT_4I and FT_3I levels were decreased and this was not accompanied by an increase in serum TSH-level could indicate pituitary insufficiency, but no other tests on pituitary function were carried out.

The demonstration of an increased frequency of thyroid autoantibodies in the present study confirms previous findings where 40% of patients with PPP had these antibodies.[6] The main discrepancy is the difference in frequency of thyroglobulin autoantibodies, 6 and 27% respectively, and this is most likely explained by differences in the methods used.[11] The finding of increased thyroid size, increased frequency of thyroid autoantibodies and a slight decrease in thyroid hormones is compatible with autoimmune thyroid disease—Hashimoto's thyroiditis. It is well known that these patients frequently develop hypothyroidism.[21] No attempt was made to confirm the diagnosis of thyroiditis by biopsy of the gland. Twelve of 44 (27%) patients could not be investigated for a variety of reasons. Perhaps this possible selection bias affected the frequency of thyroid disease in our study.

In conclusion, PPP is associated with an increased frequency of goitre and thyroid autoantibodies and with a decrease in the serum level of thyroid hormones compatible with autoimmune thyroid disease. Long-term follow-up of larger groups will possibly clarify whether a significant number develop symptomatic thyroid disease and whether PPP investigation of thyroid function should be necessary.

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A double-blind, placebo-controlled trial of topical 1,25-dihydroxycholecalciferol in psoriasis

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SUMMARY

In a double-blind trial of topical 1,25-dihydroxycholecalciferol in 47 patients with psoriasis no benefit was shown compared with placebo.

Recent research has shown that epidermal keratinocytes and dermal fibroblasts carry a receptor for 1,25-dihydroxycholecalciferol[1] and that physiological concentrations of the vitamin regulate terminal differentiation and suppress proliferation of the keratinocytes.[2] This has prompted several open studies using oral and topical vitamin D_3 analogues (1αhydroxycholecalciferol, 1,25-dihydroxycholecalciferol and 1,24-dihydroxycholecalciferol) in patients with psoriasis.[3,4] We have compared the effect of topical 1,25-dihydroxycholecalciferol in a medium-chain triglyceride (MCT) base, with that of the MCT base alone in a double-blind, placebo-controlled trial.

METHODS

Forty-seven patients with chronic stable psoriasis entered the trial. Twenty-eight were male and 19 female. The average age was 40 (range 18–70). Two equivalent psoriatic plaques for each patient, less than 30 mm in diameter were selected for treatment. One plaque was treated twice daily with 0·5 μg 1,25-dihydroxycholecalciferol in 0·25 ml base for 4 weeks and the other was treated with the base alone. The plaques were assessed before and after the trial period by the same examiner. Erythema, scaling and induration were scored on an arbitrary scale of 1–4. None of the patients were on systemic or topical treatments for the 2 weeks prior to starting the study apart from emollients which they continued to use during the trial. Full blood count,

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erythrocyte sedimentation rate, plasma calcium, phosphate, liver function tests and plasma lipids were estimated before and after the trial. A routine urinalysis was performed.

Analysis

The total score (maximum 12) for each psoriatic plaque, before and after treatment, was calculated and the change between the actively-treated and the placebo-treated plaque compared. The result was then analysed using the Wilcoxon signed rank test for non-parametric data. This was repeated using the individual scores for erythema, scaling and induration, before and after treatment. The mean total score was calculated by adding the individual total scores and averaging the results before and after treatment. This was repeated for the individual scores of erythema, scaling and induration.

RESULTS

Forty-one patients completed the trial. Five patients failed to attend follow-up for social reasons and one considered that emollients were inadequate treatment for the psoriasis. There were no significant adverse effects. The blood and urine tests showed no significant changes. The plasma lipid results showed some abnormalities before and after treatment but there were no changes with treatment and the abnormalities were likely to be due to the taking of blood samples from non-fasting patients.

Twenty-one of the patients showed a greater improvement on the actively-treated plaque as compared to 10 on the placebo-treated plaque. Using Wilcoxon test this difference was not significant ($P = 0{\cdot}230$). The results for the total mean scores are summarized in Figure 1 and the results for the individual mean scores for erythema, scaling and induration are shown in Figure 2. Table 1 shows the number of patients who improved by 0–25, 26–50, 51–75 and 76–100% on each treatment.

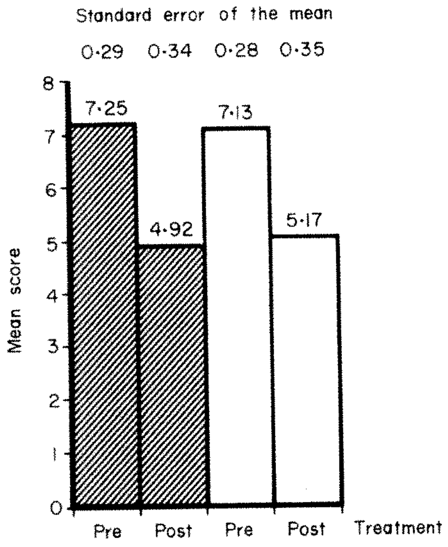

FIGURE 1. Total mean scores for psoriatic plaques on placebo (□) and 1,25-dihydroxycholecalciferol treatment (■).

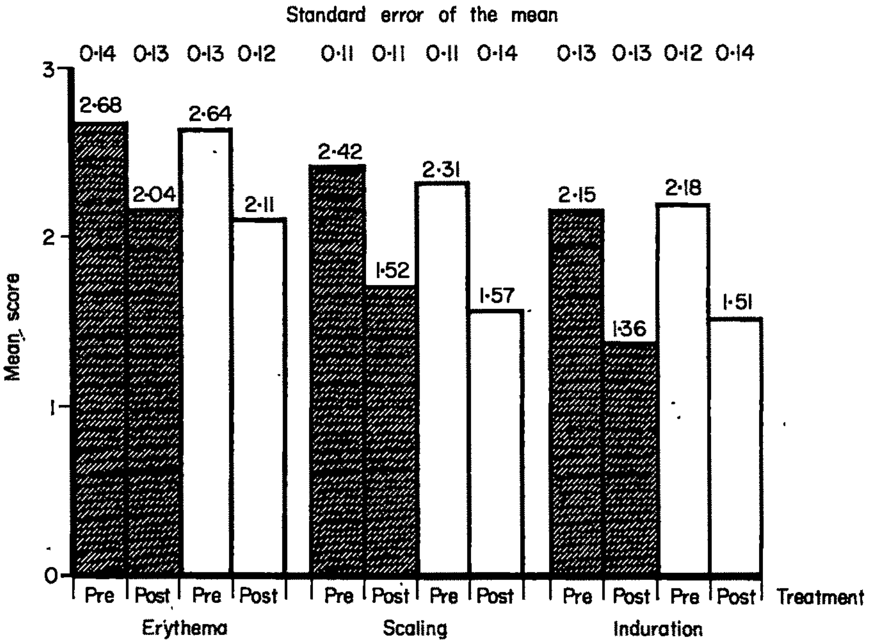

FIGURE 2. Mean scores for erythema, scaling and induration of psoriatic plaques on placebo (□) and 1,25-dihydroxycholecalciferol treatment (▨).

TABLE 1. Number of plaques which improved by 0–25, 26–50, 51–75, 76–100%, deteriorated or showed no change, according to treatment.

	Deterioration in plaque	No Change in plaque	0–25% improvement	26–50% improvement	51–75% improvement	76–100% improvement
1,25-Dihydroxycholecalciferol treatment	4	3	10	16	9	0
Placebo treatment	0	6	15	15	5	1

DISCUSSION

In this study we have not demonstrated a significant effect of topical 1,25-dihydroxycholecalciferol compared to the placebo. There may be several reasons for this. The triglyceride base was very fluid and many patients found it difficult to apply the full 0·5 ml daily. Most patients probably received much less than 1 μg of the active drug on the plaque each day. Previous trials have used ointment bases with concentrations of 0·5–4·0 μg of the vitamin per gram,[3,4] and as the drug's action is known to be concentration dependent,[5,6,7] the solution used in this trial was probably inadequate. The triglyceride base dried to leave an occlusive film over the psoriatic plaque. It is possible that this had a beneficial effect on the psoriasis itself, as it has been shown that bland emollient bases can have a direct effect on the hyperproliferation of keratinocytes in psoriasis.[8] The small additional benefit of the vitamin in the base may thus have been masked.

Various modes of action of the drug have been postulated. A study of topical 1,25-dihydroxycholecalciferol failed to show any effect on epidermal cell cycle kinetics.[9] This suggests that immune modulation may be responsible, as 1,25-dihydroxycholecalciferol has detectable effects on lymphocytes *in vitro*.[10] Other possible modes of action in psoriasis are deficient receptor expression in fibroblasts[6] and keratinocytes[11] which varies with the differentiation of these cells.

1,24-dihydroxycholecalciferol (MC903) has been reported to be as active as the 1,25 isomer[12] in tumour cell lines, although having less effect on calcium metabolism. A recent study has shown MC903 to be effective in psoriasis,[7] although only at high concentrations. This vitamin D_3 analogue was considered to be a safe topical treatment for psoriasis.

Further investigation of topical vitamin D_3 and its analogues may still be worthwhile, particularly when used in combination with other treatments or in irritated psoriasis not tolerating conventional therapies.

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A 4% erythromycin and zinc combination (Zineryt®) versus 2% erythromycin (Eryderm®) in acne vulgaris: a randomized, double-blind comparative study

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SUMMARY

A double-blind, randomized multi-centre study was performed to evaluate the efficacy of a 4% erythromycin and zinc combination (Zineryt®) versus 2% erythromycin (Eryderm®). One-hundred and twenty-two patients suffering from acne vulgaris were treated with either Zineryt® lotion or 2% erythromycin lotion. Acne grading and lesion counts for comedones, papules, pustules, nodules and macules were performed at each visit at 0, 1, 2, 4, 8 and 12 weeks. Treatment with Zineryt® lotion was found to be more effective than with 2% erythromycin as regards the reduction in number of the acne lesions and the severity grade of the acne.

The current treatment for acne vulgaris varies and the most widely used drugs are benzoyl peroxide, retinoids, hormones and antibiotics.[1] Topically applied antibiotics produce fewer conclusively systemic side-effects than when given systemically. They have not been shown to give rise to a greater incidence of bacterial resistance than when given systemically. Topical erythromycin formulations are now available in most countries, usually as 1·5 or 2% solutions and are just as effective as 1% clindamycin in the treatment of acne vulgaris.[2,3]

Zineryt® contains the antibiotic erythromycin (4%) in a zinc acetate complex in a di-isopropyl-octane-dicarboxylate ethanol solution. This combination has been proved to be effective in the treatment of acne vulgaris[4] and superior to 1% clindamycin lotion.[5] In order to evaluate the possible beneficial effects of the erythromycin (4%) and zinc combination (Zineryt®) in the treatment of acne, a randomized, double-blind study was conducted comparing Zineryt® with a commercially available lotion preparation of 2% erythromycin without zinc (Eryderm®).

METHODS

Patients were recruited in two centres in The Netherlands and one centre in Belgium. All patients had moderate to severe acne vulgaris (mean grade 3·7) and the age range was 14–41 years (mean 22 years). No patient had been on any topical acne therapy for at least 2 weeks before entering the study, and had not had any oral anti-acne preparation in the previous 3 months. Each patient was randomly allocated to one of two treatments. A third person supplied the allocated medication to the patient who was instructed to apply the lotion to the affected areas twice daily, the bottle being held in a slanting position in order to wet the applicator, which was moved gently over the skin.

A count of the lesions on the face was performed at the beginning of the study and after 1, 2, 4, 8 and 12 weeks of treatment. The following parameters were scored at each assessment: acne grade on a 0–8 scale,[6] and number of comedones, papules, pustules, nodules and macules.[7]

In order to check whether the patients in the two treatment groups were comparable in respect of all of the above mentioned parameters their distribution was analyzed using the Kruskal–Wallis test. No statistically significant differences were found in this respect between the two groups ($P > 0{\cdot}1$).

The efficacy of both treatments was compared by evaluating the changes in lesion counts at each subsequent visit as compared to the baseline level ($\alpha = 0{\cdot}05$ two-sided, Mann–Whitney *U*-test). Data were also analysed as percentages of the pre-treatment numbers. Since acne vulgaris is a cosmetically hampering disease especially in view of the inflamed lesions (papules and pustules), these have also been looked at as a separate variable in the analysis of the study.

Adverse events were recorded and their relevance assessed. If, in the opinion of the investigator, the adverse effect was greater than the potential therapeutic benefit, the medication was discontinued.

RESULTS

Data were obtained from a total of 122 patients. All patients complied with the inclusion criteria except for 14 patients with only mild acne (severity rating 1 or 2), eight of these patients being assigned to treatment with Zineryt® lotion and six to treatment with erythromycin lotion. A total

TABLE 1. The mean values for acne severity and lesion counts at the beginning of the study and after 12 weeks of therapy with Zineryt® or erythromycin 2% lotion

	Zineryt® mean		Erythromycin mean	
	week 0	week 12	week 0	week 12
Acne severity grade	3·6	1·6	3·9	2·8
Comedones	50·7	16·5	54·0	27·3
Papules	21·0	3·6	22·1	10·2
Pustules	10·9	0·6	13·0	3·9
Inflamed lesions	31·9	4·2	35·1	14·1
Nodules	0·5	0·0	0·5	0·2
Macules	8·6	5·5	8·5	8·5

TABLE 2. Results of the testing procedures, as summarized by the respective P-values, on the results after 1 and 12 weeks of therapy with either 2% erythromycin (ERY) or Zineryt® (ZIN). (The acne severity grade and the number of nodules were not calculated as percentages because their values were too low)

		Changes from pre-treatment level			
		absolute reduction		relative reduction	
	Week	ZIN	ERY	ZIN	ERY
Acne grade	1	<0·05	<0·05		
	12	<0·001	<0·001		
Comedones	1	<0·001	<0·001	<0·001	<0·001
	12	<0·001	<0·001	<0·001	<0·001
Papules	1	<0·001	<0·01	<0·001	<0·001
	12	<0·001	<0·001	<0·001	<0·001
Pustules	1	<0·001	<0·001	<0·001	<0·001
	12	<0·001	<0·001	<0·001	<0·001
Total of inflamed lesions	1	<0·001	<0·001	<0·001	<0·001
	12	<0·001	<0·001	<0·001	<0·001
Nodules	1	<0·01	>0·20		
	12	<0·01	>0·20		
Macules	1	<0·01	>0·10	<0·05	>0·20
	12	<0·01	>0·20	<0·001	<0·001

of 20 patients did not complete the full 12 weeks treatment because they dropped out of the trial or were withdrawn (two in each treatment group).

The average lesion counts per treatment group at the beginning of the study and after 12 weeks of therapy are listed in Table 1. Both Zineryt® and erythromycin lotion gave a statistically significant reduction (Table 2) of acne grade, comedones, papules and pustules at each subsequent visit ($P < 0{\cdot}001$). The reduction of nodules and macules was statistically significant only in the Zineryt® group ($P < 0{\cdot}01$). As shown in Figures 1–4, the improvement of the acne lesions was more marked in the Zineryt® group than in the erythromycin group during the entire course of the study. These differences between the two treatment groups were statistically significant in favour of Zineryt® for both the absolute and relative (%) reduction of nearly all parameters at the final assessment. As can be seen from Figure 4, the mean relative reduction of inflamed lesions was 85% for Zineryt® and 46% for 2% erythromycin. A quicker onset of action for Zineryt® is shown by the significantly better relative reduction for papules and total inflamed lesions (papules plus pustules) at weeks 1, 2, 4, 8, and 12 as compared to 2% erythromycin. A striking difference between the two treatments was in the counts of pustules. Whereas analysis of the absolute data revealed no significant differences between the treatments early on, there was a significant difference between the percentage values in favour of Zineryt® lotion after 8 and 12 weeks of therapy (Fig. 3).

Dryness of the skin, itching, burning sensation and/or erythema were reported as side-effects in 13 of the patients treated with Zineryt® and in 8 patients treated with 2% erythromycin. These symptoms were generally mild, only two patients in both groups needed to be withdrawn from the study.

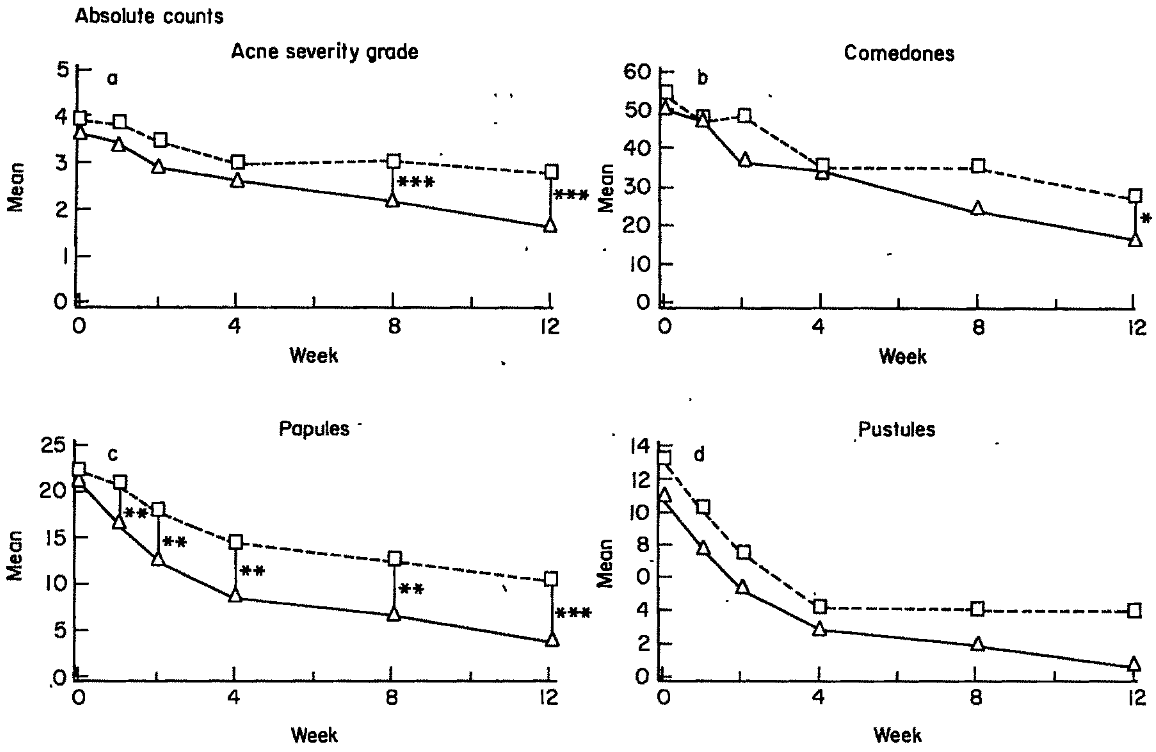

FIGURE 1. (a) Improvement of mean acne severity grade during 12 weeks of therapy with either Zineryt® lotion or 2% erythromycin lotion. Statistically significant differences between treatments are found after 8 and 12 weeks of therapy. (b) Improvement of mean number of comedones during 12 weeks of therapy with either Zineryt® lotion or 2% erythromycin lotion. A statistically significant difference between treatments is found after 12 weeks of therapy. (c) Improvement of mean number of papules during 12 weeks of therapy with either Zineryt® lotion or 2% erythromycin lotion. Statistically significant differences between treatments are found after 1, 2, 4, 8 and 12 weeks of therapy. (d) Improvement of mean number of pustules during 12 weeks of therapy with either Zineryt® lotion or 2% erythromycin lotion. No statistically significant differences between treatments are found. -□---□-, 2% erythromycin; -△——△- Zineryt®. $^{***}P < 0\cdot001$, $^{**}P < 0\cdot01$, $^{*}P < 0\cdot05$.

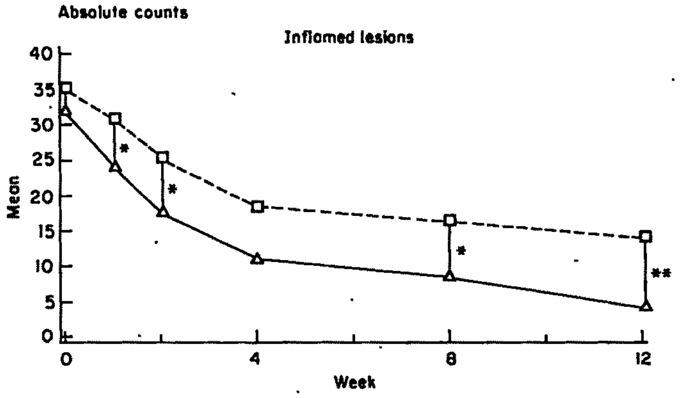

FIGURE 2. Improvement of mean number of inflamed lesions during 12 weeks of therapy with either Zineryt® lotion or 2% erythromycin lotion. Statistically significant differences between treatments are found after 1, 2, 8 and 12 weeks of therapy. -□---□-, 2% erythromycin; -△——△-, Zineryt®. $^{**}P < 0\cdot01$, $^{*}P < 0\cdot05$.

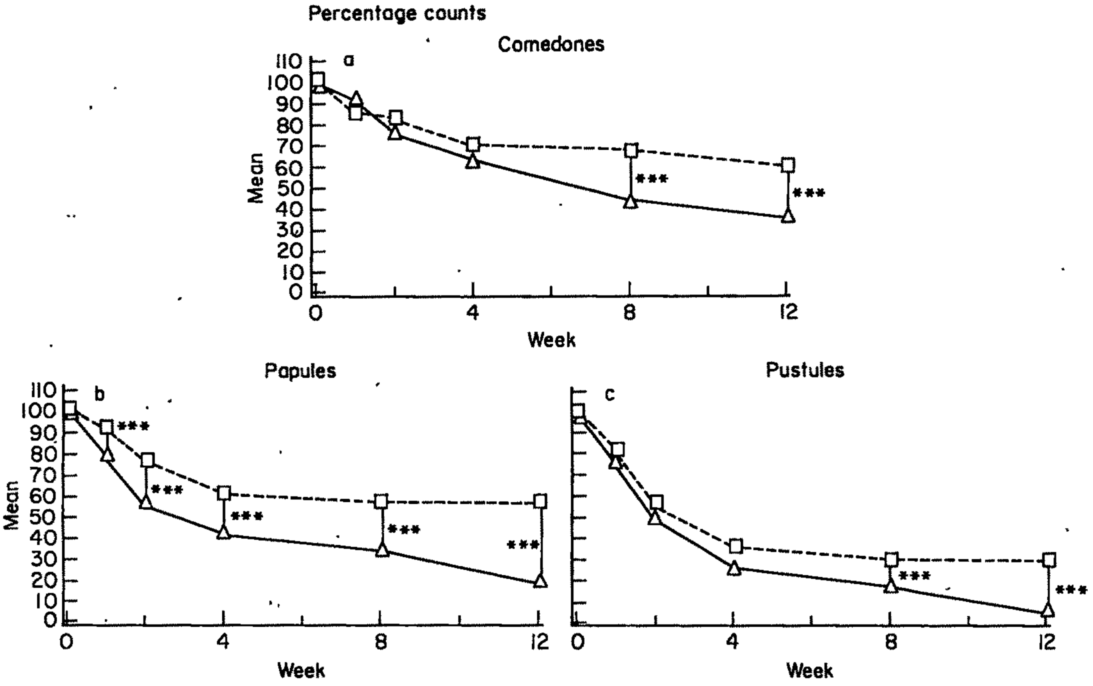

FIGURE 3. (a) Percentage reduction of number of comedones during 12 weeks of therapy. Statistically significant differences between treatments are found after 8 and 12 weeks. (b) Percentage reduction of number of papules during 12 weeks of therapy. Statistically significant differences between treatments are found after 1, 2, 4, 8 and 12 weeks. (c) Percentage reduction of number of pustules during 12 weeks of therapy. Statistically significant differences between treatments are found after 8 and 12 weeks. -□ - - □-, 2% erythromycin; -△——△-, Zineryt®. $P < 0{\cdot}001$.

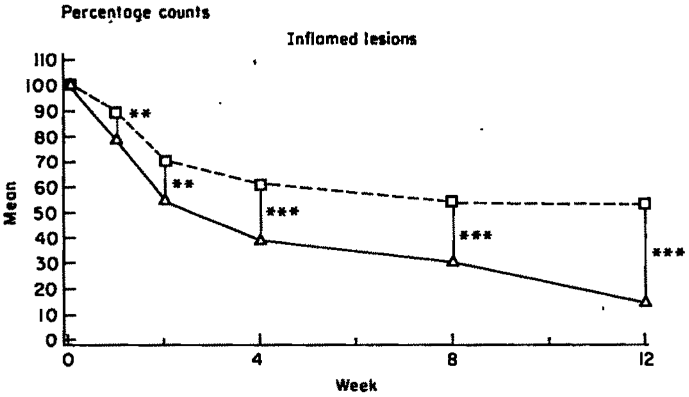

FIGURE 4. Percentage reduction of number of inflamed lesions during 12 weeks of therapy. Statistically significant differences between treatments are found after 1, 2, 4, 8 and 12 weeks. -□ - - □-, 2% erythromycin; -△——△-, Zineryt®. $***P < 0{\cdot}001$, $**P < 0{\cdot}01$.

DISCUSSION

The present study has shown conclusively that Zineryt® is more effective in the treatment of acne vulgaris than a 2% erythromycin lotion. The superiority of Zineryt® in the treatment of acne vulgaris could be due to several factors. Firstly, the concentration of erythromycin (4%) is higher than that in the 2% reference medication, and the increased benefit could simply represent a dose-response difference. Secondly, the zinc acetate complex may enhance the penetration of the erythromycin in the skin.[4] A recent pilot study has shown that Zineryt® tends to be more effective in reducing the bacterial flora of the pilosebaceous duct (*Micrococcae* and *Propionibacterium acnes*) than 2% erythromycin (unpublished data on file, Gist-brocades, Delft, The Netherlands). Thirdly, zinc itself could play a role in the superior efficacy of Zineryt®. In a recent *in vitro* study (unpublished data on file, Gist-brocades) the MIC of erythromycin and zinc on five follicular isolates of *P. acnes and Staphylococcus epidermidis* was determined. In this study zinc alone inhibited both the erythromycin sensitive and the erythromycin resistant strains tested, and the resistant bacteria proved to be even two to four times more sensitive to the zinc than the erythromycin-sensitive strains. This could explain the better clinical efficacy of Zineryt®.

The di-isopropyl-octane-dicarboxylate ethanol-containing vehicle could also have an additional beneficial effect on the skin in the treatment of acne vulgaris. All these factors could explain why Zineryt® lotion was more effective in the treatment of acne vulgaris than 2% erythromycin.

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Variegate porphyria associated with hepatocellular carcinoma

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SUMMARY

A case is described of hepatocellular carcinoma presenting with the cutaneous and biochemical features of variegate porphyria.

There are well established associations between hepatocellular carcinoma and both porphyria cutanea tarda[1–4] and acute intermittent porphyria.[5–7] We report a case in which the diagnosis of hepatoma was heralded by the development of the cutaneous manifestations of variegate porphyria.

CASE REPORT

A 70-year-old Caucasian female presented in the spring of 1987 with a 9-month history of a blistering eruption affecting the backs of the hands, the forehead, nape of neck and helices of ears. Her general health was good, and she took no medications or dietary supplements. She abstained from alcohol and there was no history of jaundice, hepatitis or symptoms that could be attributable to acute porphyric attacks. There was no family history of skin or liver disease. Examination of the affected regions revealed superficial scarring, tense blisters, scabs and milia. Her liver was firm and enlarged, but there were no other stigmata of chronic liver disease. Histological examination of fresh blisters showed subepidermal separation with 'festooning' of the floor, and prominent periodic acid Schiff-positive material around the dermal blood vessels. Direct immunofluorescence examination of perilesional skin showed perivascular deposition of IgM, IgA, C3 and fibrin. Screening for porphyria was negative on two occasions. Liver function tests showed moderate elevations of alkaline phosphatase and aspartate aminotransferase. Serological testing for hepatitis B surface antigen was negative, and an autoantibody profile revealed the presence of smooth muscle antibodies. Serum iron and ferritin levels were normal.

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TABLE 1. Porphyrin analysis

	Patient	Reference range
Urine		
Total porphyrin (μg/l)	* 567	20–210
Porphobilinogen (mg/l)	0·1	0–2
Faeces		
Coproporphyrin (μg/g dry wt)	142	0–30
Protoporphyrin (μg/g dry wt)	257	0–125
Ether-insoluble ('x') porphyrin (μg/g dry wt)	298	0–20

* Mainly coproporphyrin.

Despite the negative porphyrin screens, a presumptive diagnosis of porphyria cutanea tarda was made, and she was treated with hydroxychloroquine. Therapy was discontinued after 6 days because of gastrointestinal side-effects. Shortly afterwards she was admitted as an emergency with low right-sided pleuritic-type pain. An ultrasound examination of the abdomen showed a large echogenic mass in the right lobe of the liver, with extension into the left lobe. Histological examination of the liver mass confirmed it to be a well-differentiated hepatocellular carcinoma. The uninvolved liver was not biopsied. There had been no deterioration of the liver function tests. The serum level of α-fetoprotein was greater than 400 kU/l (normal < 8 kU/l). Subsequent quantitative porphyrin estimations demonstrated a pattern characteristic of variegate porphyria[8] (Table 1), with markedly elevated faecal levels of protoporphyrin, coproporphyrin and ether-insoluble ('x') porphyrin. The plasma contained large amounts of porphyrin with a maximum emission at 620 nm, possibly reflecting the presence of a mixture of coproporphyrin (maximum emission 615 nm) and the porphyrin–protein complex (maximum emission 625 nm) that is present in variegate porphyria.[9]

Computerized axial tomography of the abdomen and a bone scan showed no evidence of extrahepatic spread, but, in view of her age and the extent of liver involvement by the tumour it was decided that she would have chemotherapy rather than removal of the tumour. She was unable to tolerate adriamycin therapy and her condition deteriorated and she died 7 months after presentation.

DISCUSSION

In a recent study of the cause of death in 82 patients with acute hepatic porphyria in Finland,[10] one of the seven patients with hepatocellular carcinoma had variegate porphyria. Other than this case, variegate porphyria does not appear to have been previously reported in association with hepatocellular carcinoma. Hepatomas occurring in patients with porphyria cutanea tarda almost invariably arise within a cirrhotic liver.[11] Variegate porphyria, in contrast to porphyria cutanea tarda, is not normally associated with liver dysfunction. This patient had no apparent risk factors for the development of hepatoma. The presence of circulating smooth muscle antibodies, however, raises the possibility that she may have had subclinical chronic active hepatitis, but, unfortunately, the histological state of the non-tumorous liver in our case is not known.

The most probable relationship between hepatoma and variegate porphyria in this patient is that she carried the gene for variegate porphyria, but had remained latent until a mild degree of intrahepatic cholestasis, consequent to the enlarging coincidental tumour, resulted in diminished biliary excretion of porphyrins and precipitated the cutaneous symptoms. Drug-induced cholestasis has been reported to provoke cutaneous symptoms in previously asymptomatic carriers of the gene for variegate porphyria.[12]

A less likely possibility is that the hepatocellular carcinoma was a porphyrin-secreting tumour and that the expression of variegate porphyria was a true paraneoplastic phenomenon. Porphyrin-secreting hepatomas producing a clinical and biochemical picture resembling porphyria cutanea tarda have been reported,[2,13] but are rare. Although the pattern of porphyrin excretion in these cases has not always been typical of porphyria cutanea tarda, none have shown the features of variegate porphyria. Demonstration of a low protoporphyrinogen oxidase activity in lymphocytes or skin fibroblasts would have confirmed the diagnosis of variegate porphyria in our patient but we were unable to carry out this investigation before her rapid deterioration.

It remains to be seen whether the association of primary liver cancer with acute intermittent porphyria can be extended to include the other, less common, types of acute hepatic porphyria such as variegate porphyria and whether there is an increased risk amongst individuals with latent porphyria.

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Extensive skin ulceration due to EF-4 bacterial infection in a patient with AIDS

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SUMMARY

We report an infection by EF-4 bacteria in an AIDS patient.

Eugonic fermenting bacteria (EF-4) were first described by Tatum *et al.*[1] and though this pasteurella-like group is as yet unclassified, its biochemical and morphologic features have been documented.[2,3] Though frequent in the oral and nasal fluid of dogs,[2,4] EF-4 bacteria have been rarely reported in human pathology and have only been isolated from skin abscesses caused by animal bites.[1,3,5,6] We describe a patient in whom a very severe skin infection, probably due to EF-4, led to the discovery that he had the acquired immunodeficiency syndrome (AIDS).

CASE REPORT

A 27-year-old male former drug addict presented with multiple extensive painful ulcerations on the scrotum, thighs (Fig. 1) and perianal area. These lesions had appeared 3 weeks earlier and spread rapidly. Clinical examination was otherwise normal. ELISA assays and Western blotting for HIV were positive and immunological assessment showed a decreased white blood cell count (1400 WBC/mm^3, 310 neutrophils/mm^3 and 610 lymphocytes/mm^3) and a CD4$^+$ cell count of 90/mm^3. Skin tests with tuberculin and *Candida* were negative. Thrombocytopenia of 99 000/mm^3 was noted and a lupus-type circulating anticoagulant was detected. Blood cultures were negative. Bronchioalveolar lavage revealed *Pneumocystis carinii* cysts and *Candida albicans.* Serum tests for toxoplasmosis and hepatitis B were positive. Tests for herpes virus and cytomegalovirus were positive. The VDRL, TPHA and FTA tests were negative.

A biopsy and multiple swabs were taken from the lesions. The histology showed necrotic ulceration but neither fungus nor the typical cytopathic changes due to herpes virus or cytomegalovirus infection were observed. Tzanck smear test, dark field examination, culture for

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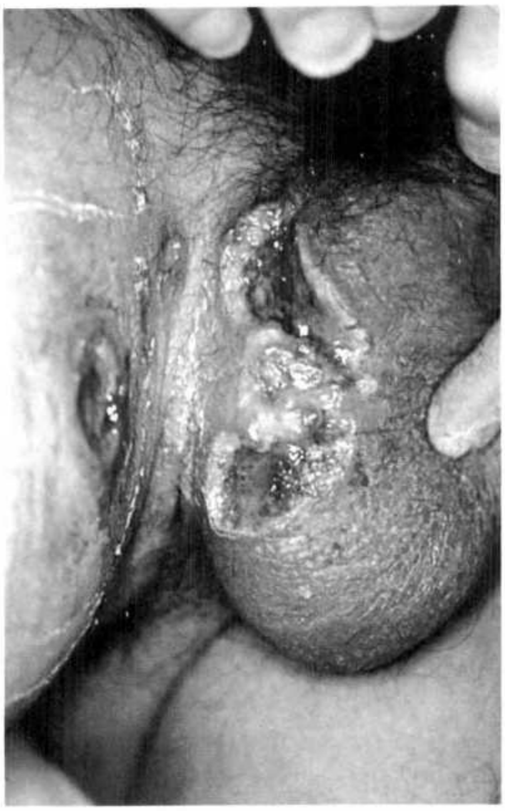

FIGURE 1. Extensive ulceration located on the inner thigh and the scrotum.

fungus, mycobacteria, chlamydia and herpes virus were all negative. On Gram staining swabs taken from the ulcers showed numerous short Gram-negative bacteria that were identified as a group EF-4 bacterium. Cultures were performed using chocolate (heated blood) agar (Bio-Merieux, Marcy l'Etoile, France) and Schaedler broth (Bio-Merieux, France) for 2 days at 37°C in a CO_2-enriched atmosphere. The biochemical and cultural characteristics of the stain were the same as previously studied EF-4 strains.[3,7] (Table 1). On the basis of evidence showing a lack of arginine dihydrolase, gelatinase and gas production from nitrate, the organism was classified in biogroup b.[3] The strain was found to be sensitive to aminoglycosides, penicillins, second and third generation cephalosporins, macrolides, fosfomycin, rifamycin, imipenem, systemic quinolones, and co-trimoxazole.

Several swabs were taken from the patient's dog and group EF-4 bacteria were isolated from oral and nasal fluids. They had the same properties as the bacteria obtained from the patient's lesions and the endonuclease restriction patterns and whole cell protein polyacrylamide gel electrophoresis were identical.

The patient was treated for both the pneumocystic infection and the skin ulcers with sulphamethoxazole 1600 mg/day and trimethoprim 320 mg/day, and the cutaneous lesions stopped spreading and the ulcers healed completely within 3 weeks.

TABLE 1. Biochemical characteristics of EF-4 group bacteria

Test	Published data EF-4 biogroup b	Reaction of present strain
Oxidase	+	+
Catalase	+	+
Motility	−	−
Arginine dihydrolase	−	−
Lysine decarboxylase	−	−
Ornithine decarboxylase	−	−
Urease	−	−
Gelatin hydrolysis	−	−
Nitrate reduction to nitrite	+	+
Gas from nitrate	−	−
Acid production from		
D-glucose	+	+
D-mannose	−	+
maltose	−	−
ribose	+	+
Tetrathionate reduction	+	+
Resistance to 0/129 agent	+	+
Yellow pigment	+/−	+

DISCUSSION

Bacterial skin infections are common in AIDS patients[8] and while staphylococcal infections are most common, other bacteria such as *Pseudomonas* spp., *Hemophilus influenzae*,[8] or *Rhodococcus equi*[9] have been observed, but this is the first reported case involving a pasteurella-like group EF-4 bacterium.

In AIDS patients, herpes simplex often results in extensive areas of painful ulceration[10] very similar to the lesions described here, but ulcerated skin or mucosal lesions have been reported with infections due to *Cryptococcus neoformans*,[11] *Staphylococcus aureus*,[8] *Pseudomonas* spp.,[8] *Cytomegalovirus*,[12] mycobacteria[8] and syphilis.

It is likely that EF-4 was the main cause of the severe perineal ulcerations in this patient. No other infectious agent was detected and treatment with antibiotics had a dramatic effect. Although no cytopathic histologic changes were detected and viral cultures were negative, there is the possibility that a herpes simplex lesion became secondarily infected by the group EF-4 bacteria. Indeed, skin lesions produced by more than one infectious agent including two virus infections,[13] fungal and viral infections[14] and mixed viral and bacterial infections[10] are frequent in patients with AIDS. It is probable that our patient acquired the EF-4 bacterial infection from his dog and the same strain of organism was isolated from the mouth of this animal. Though he was not bitten by the dog it is likely that our patient scratched himself in the groin after his hands had been licked by this animal and as a consequence developed these infected ulcers. The marked immune deficiency from HIV infection was probably responsible for this unusual opportunistic bacterial infection.

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Cutaneous focal mucinosis

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SUMMARY

A 39-year-old man had typical cutaneous focal mucinosis on the left forearm. The fibroblasts in the myxomatous lesion had many cytoplasmic vesicles, and on electron microscopy, these were compatible with condensing vacuoles or secretory granules. The fibroblasts in this case were considered to be mucoblasts involved in the over-production of the ground substance, that was mainly composed of hyaluronic acid.

Cutaneous focal mucinosis (CFM) was accepted as a distinct clinical entity,[1] but recently was included in the superficial angiomyxomas.[2] Clinically, it is characterized by a solitary, asymptomatic, flesh-coloured white papule or nodule which occurs on the face, trunk or extremities. On histology there is a localized accumulation of mucinous material in the dermis with an increase in the number of fibroblasts. There have been only a few electron microscopic studies on CFM.[3,4] We report a typical case of CFM with the histological findings on light and electron microscopy.

CASE REPORT

A 39-year-old man was seen in February 1987 and had an asymptomatic, flesh-coloured papule that developed on the left forearm over the previous 7 months. The lesion slowly enlarged, and there was a solitary, flesh-coloured, hard nodule on the extensor aspect of his left forearm. The surface of this nodule was smooth and it measured 1·2 x 1·2 cm in size (Fig. 1a). All routine laboratory investigations, including thyroid function tests, were normal.

Light microscopy

There was a localized myxomatous lesion that extended from the papillary to the deeper part of the dermis. The margins of the lesion were not sharply delineated and blended gradually with the surrounding normal connective tissue. The myxomatous lesion consisted of a large amount of ground substance wih infiltrating cells, collagen bundles, and blood vessels. Of these, the increased amount of ground substance was the most striking feature (Fig. 1b). It was slightly basophilic with haematoxylin and eosin and positively stained with colloidal iron and was

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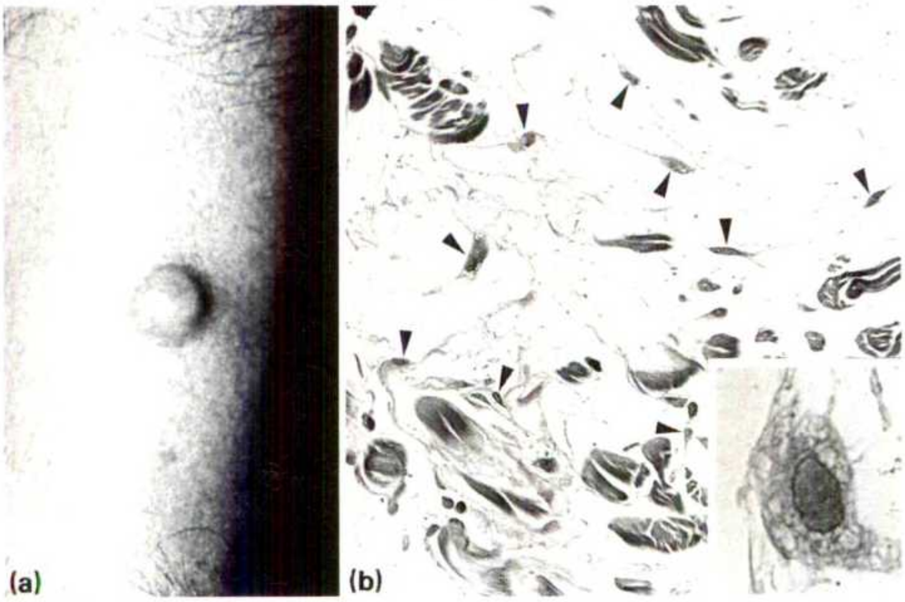

FIGURE 1. (a) Appearance of the lesion on the extensor aspect of the left forearm. (b) Fibroblasts (arrowheads) with thin elongated cytoplasm in the ground substance (H&E × 200). Insert—many vesicles in the cytoplasm of the fibroblasts (H&E × 990).

negative following hyaluronidase digestion. The material was positive with alcian blue at pH 4·0 but not at pH 1·5. It was negative on PAS staining. These results indicated that the ground substance was mucin rich in hyaluronic acid. The cells seen consisted mainly of fibroblasts, plasma cells and lymphocytes. The fibroblasts were slightly increased in number and were diffusely scattered in the nodule (Fig. 1b). They were oval, spindle or stellate in shape, often with very long cytoplasmic processes and with prominent nuclei. A characteristic feature was that the cytoplasm of these fibroblasts contained many vesicles (Fig. 1b insert). The collagen bundles appeared to be reduced in number and were widely separated by the mucin. The blood vessels appeared normal. There was a band of normal connective tissue between the epidermis and the myxomatous lesion. The overlying epidermis was acanthotic and the basal layer was pigmented.

Electron microscopy

There were electron-lucent areas in which the cellular and fibrous elements were sparsely scattered (Fig. 2) and in which the ground substance was present. Spindle or stellate cells were frequently seen (Fig. 2a) and most had a few, very thin elongated cytoplasmic processes (Fig. 2b). The nuclei were oval or irregular in shape, with abundant euchromatin. The cytoplasm of these cells contained many large vacuoles, with a well-developed rough endoplasmic reticulum and numerous ribosomes. These cells lacked a basal lamina. They were identified as fibroblasts (Fig. 3).

The large vacuoles were round or oval in shape with a distinct outer limiting membrane. Some resembled the dilated rough endoplasmic reticulum with attached ribosomes on the outer limiting membrane. They varied in size approximately from 0·5 to 1·5 μm in diameter. They had a tendency to fuse with each other (Fig. 2a insert). The contents of the vacuoles were moderately electron dense and finely granular. There were one or two vacuoles present in the long slender

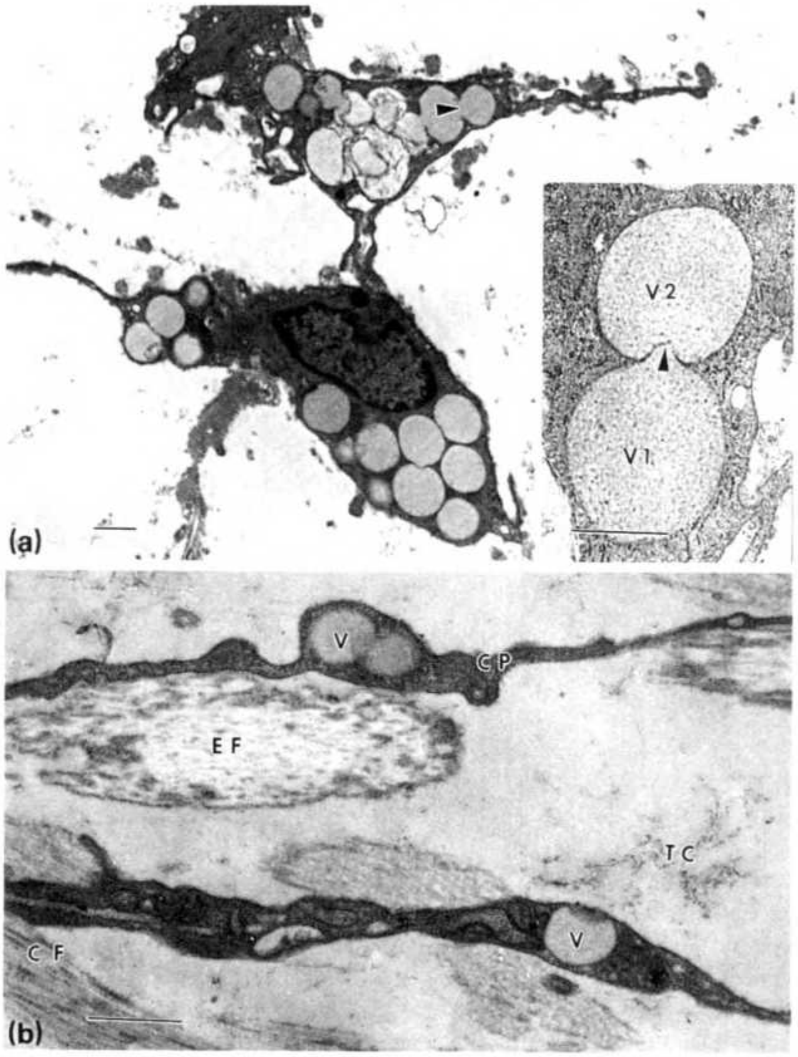

FIGURE 2. (a) Two fibroblasts with thin elongated cytoplasmic processes in the ground substance which contain several vacuoles or secretory granules surrounded by an outer limiting membrane. An arrowhead indicates the fusing portion of two vacuoles (Bar = 1 μm; × 6900). Insert—contact of the limiting membranes of two vacuoles, with one (V1) projecting into another (V2) (Bar = 1 μm; × 17 000). (b) Vacuoles or secretory granules in the slender cytoplasmic processes of fibroblasts. CF, collagen fibre, CP, cytoplasmic process, EF, elastic fibre. TC, tropocollagen, V, vacuole (Bar = 1μm, × 16 000).

cytoplasmic processes (Fig. 2b). Although the cytoplasmic processes were often in contact with each other, they did not form any junctional complexes. The fibrous elements included collagen, elastic fibrils and tropocollagen fibrils. None of these fibrous elements were increased, and the collagen and elastic fibrils were frequently in association with the cytoplasmic processes of the fibroblasts (Fig. 2b). The tropocollagen fibrils were often aggregated near the cell bodies of the fibroblasts.

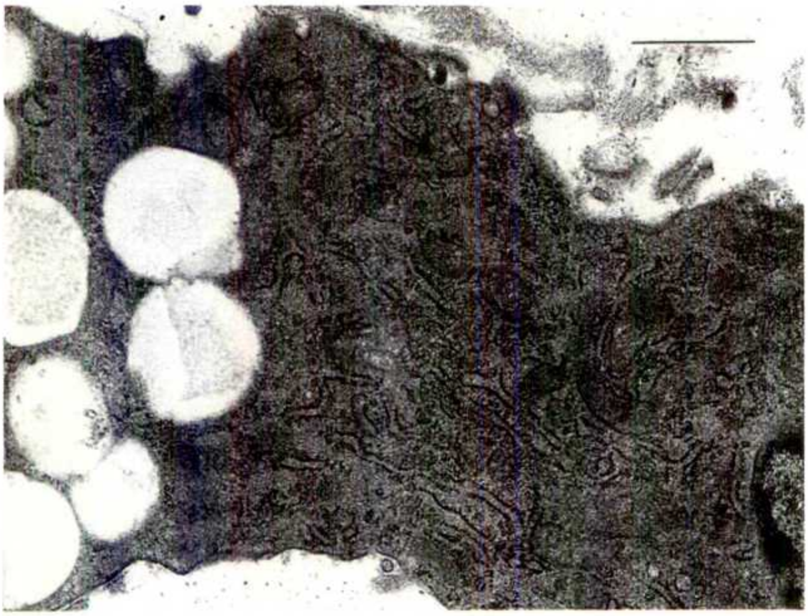

FIGURE 3. Higher magnification of a fibroblast. Many vacuoles or secretory granules with a well-developed rough endoplasmic reticulum and mitochondria are observed in the cytoplasm (Bar = 1 μm, × 21 000).

DISCUSSION

The clinical appearance and the site of the lesion on our patient was similar to those cases of CFM described by Johnson and Helwig.[1] The most striking histological features of CFM were the myxomatous matrix and proliferation of fibroblasts in the dermis.[1] The myxomatous matrix contained a large amount of mucinous ground substance, mostly hyaluronic acid as found in our case. The fibroblasts were also slightly increased and showed the characteristic cytoplasmic vesicles. These vesicles, as seen in our case, corresponded to the small globules in the cytoplasm of the fibroblasts as described by Johnson and Helwig.[1] We found on electron microscopy that these vesicles were vacuoles with a distinct outer limiting membrane and some appeared to be dilated cisternae of rough endoplasmic reticulum. Some of the vacuoles had fused with each other and most were filled with moderately electron dense and finely granular material. These ultrastructural findings are consistent with those of condensing vacuoles or secretory granules[5] and the fibroblasts in this lesion are considered to be mucoblasts as seen in pretibial myxoedema.[6]

Unlike the mucous cells that synthesize and export proteins, the fibroblasts do not commonly store their products and are therefore largely devoid of condensing vacuoles and secretory granules.[5] The main structural elements in the dermis, including collagen fibres, elastic fibres and the ground substance (an interfibrillar gel of glycosaminoglycans), are synthesized and regulated by dermal fibroblasts.[7] In this condition, the fibroblasts are mucoblasts with many large condensing vacuoles or secretory granules in the cytoplasm. This suggests that CFM is the result of a dysfunction of the fibroblasts in a localized area.

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Scleredema of Buschke associated with rheumatoid arthritis and Sjögren's syndrome

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SUMMARY

A case of scleredema of Buschke associated with rheumatoid arthritis and Sjögren's syndrome is described. The onset of the skin changes and rheumatoid arthritis was almost simultaneous and the sicca syndrome developed 4 years later.

Scleredema of Buschke is an uncommon skin disease characterized by non-pitting induration and sclerosis of the skin. It is not usually associated with other diseases, although diabetes mellitus has been noted in a significant number of patients[1] and a monoclonal gammopathy has been observed in some patients with scleredema.[2] Recently a case of scleredema was reported with rheumatoid arthritis[1] and we report a case of scleredema associated with rheumatoid arthritis and Sjögren's syndrome.

CASE REPORT

A 49-year-old female was referred to the department of dermatology in May 1988. In the previous 5 years she had joint pains and developed painless non-pruritic induration and thickening of the skin at first on the right shoulder. The skin thickening gradually extended to involve the neck, trunk, and the proximal extremities. Deformities of her joints later developed and these involved the hands, ankles and feet. Later she had dry eyes and mouth symptoms of the sicca syndrome.

Physical examination disclosed a non-pitting induration of the skin of the face, neck, shoulders (Fig. 1), trunk, and proximal extremities. The face lacked expression, with minimal wrinkles, and the patient had difficulty in opening her mouth because of the skin tightness.

The histology of a skin biopsy from the right shoulder showed no significant epidermal changes, but there was thickening of the dermis and the subcutaneous tissue was replaced by apparently swollen collagen bundles. The elastic fibres appeared normal. A colloidal iron stain showed deposition of mucin between the collagen fibres (Fig. 2) and the staining was removed

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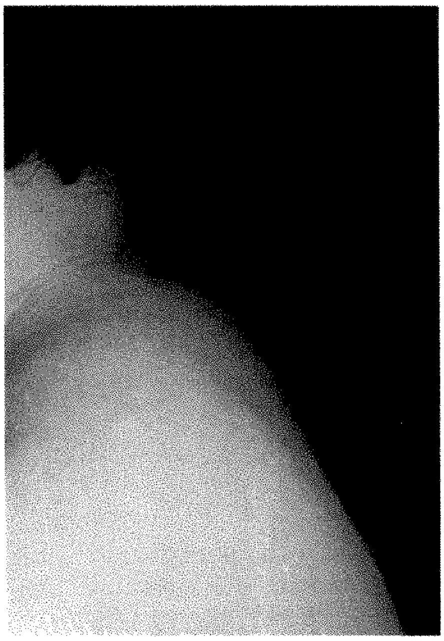

FIGURE 1. Non-pitting, solid oedema on the shoulder.

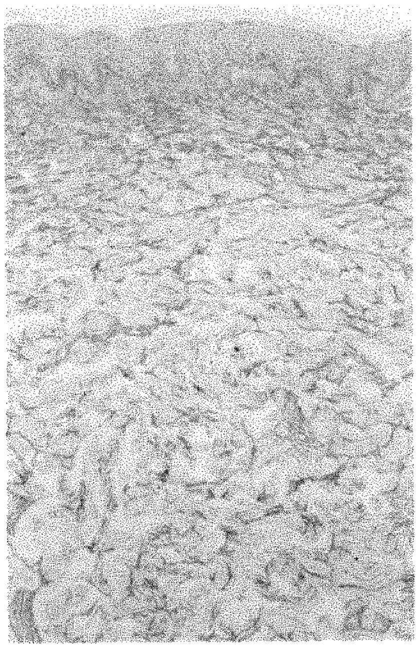

FIGURE 2. Skin biopsy section stained with colloidal iron showing deposits of mucinous material interspersed among collagen bundles throughout the thickened dermis. (magnification × 100).

with testicular hyaluronidase. No amyloid deposits were detected. Direct immunofluorescence microscopy failed to show any deposition of immunoglobulins or complement in the lesional skin.

The laboratory investigations which were either negative or normal included a full blood count with differential, urinalysis and liver function tests, as well as serum electrolytes and muscle enzyme levels. The total serum protein was 8·4 g/dl with an albumin/globulin ratio of 0·9. Levels of serum IgA, IgG and IgM were elevated to 448 mg/dl (normal, 220–340 mg/dl), 2718 mg/dl (normal, 780–1450 mg/dl) and 956 mg/dl (normal, 80–140 mg/dl), respectively, but there was no evidence of a monoclonal gammopathy. The levels of serum C3, C4, and total complement were normal. Anti-ds DNA antibodies, LE cells, antistreptolysin O as well as antistreptokinase titres, C-reactive protein and serologic tests for syphilis, were all negative. The rheumatoid factors were positive and the anti-nuclear antibody test (ANA) was positive with a titre of 1:640 using HEp-2 cells as the substrate. Anti-SSA/Ro antibodies were detected by immunodiffusion at a titre of 1:80, but antibodies against SSB/La, U1RNP, Sm and Scl-70 antigens were not detected. Chest X-ray, electrocardiogram, barium swallow, pulmonary function tests, thyroid function studies and electromyogram were all normal. Eye investigations, sialography and salivary gland scanning indicated that she had the sicca syndrome. There was no evidence of diabetes mellitus as based on fasting blood glucose levels, routine urinalysis and a glucose tolerance test. X-rays of her hands, wrists and feet showed changes compatible with the diagnosis of rheumatoid arthritis.

So far there has been no resolution of her scleredema which has now lasted for about 5 years. The patient has been on oral gold therapy (auranofin 6 mg/day), loxoprofen sodium 240 mg/day and lobenzarit disodium 240 mg/day for her rheumatoid arthritis.

DISCUSSION

In 1902 Buschke[3] described a systemic disease with woody, non-pitting induration starting at the nape of the neck and spreading throughout the body, sparing only the palms and the soles of the feet. Graff[4] defined three types of scleredema: (i) a benign self limiting disorder preceded by an upper and lower respiratory tract infection; (ii) an insidious presentation with a protracted course; and (iii) scleredema associated with diabetes mellitus which was persistent. A recent review[1] of 33 cases of scleredema, with particular reference to clinical and histological findings, revealed that the course of the disease is usually prolonged. A preceding respiratory tract infection was uncommon and did not indicate a short course. Our case falls into Graff's second category with insidious onset, extended duration and no evidence of diabetes mellitus.

Although the cause and pathogenesis of scleredema is obscure, patients with scleredema may have a factor that stimulates collagen synthesis and results in dermal fibrosis. This is supported by recent *in vitro* studies demonstrating that the serum from scleredema patients can stimulate collagen production and the incorporation of radioactive sulphate in dermal fibroblast cultures.[5]

The association of a rheumatic disease with scleredema is exceedingly rare, though a patient is cited with widespread scleredema and rheumatoid arthritis.[1] Serological tests indicative of a connective tissue disorder have not been commented on in the literature. An immune response to the SSA/Ro antigen was found in systemic lupus erythematosus,[6] Sjögren's syndrome,[7,8] subacute cutaneous lupus erythematosus,[9] and neonatal lupus erythematosus.[10–12] Recently anti-SSA/Ro antibodies were implicated in the extraglandular cutaneous vasculitis associated with Sjögren's syndrome.[13] The significance of the antibodies found in scleredema remains to be elucidated.

In summary, the findings in our case suggest an association can occur between scleredema and autoimmune rheumatic diseases such as rheumatoid arthritis or Sjögren's syndrome in some patients.

ACKNOWLEDGMENTS

Supported in part by a 1988 research grant from the Japan Lydia O'Leary Foundation, Tokyo.

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Society Proceedings

Proceedings of the 244th Meeting of the Netherlands Society for Dermatology and Venereology, Maastricht, 1 October 1988

PRESIDENT: PROFESSOR W.A.VAN VLOTEN
HOST: PROFESSOR W.J.B.M.VAN DER STAAK

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SUMMARIES OF PAPERS

Evaluation of the consensus development conference on cutaneous melanoma in the Netherlands

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On 15 September, 1984, a consensus development conference on cutaneous melanoma was held in the Netherlands by CBO, the national organization for quality assurance in hospitals. The consensus statement contained guidelines on the management of patients with primary and metastatic melanoma. In 1986, the post-conference compliance of these guidelines among dermatologists was analysed and 383 histopathological reports received by 19 departments of pathology were compared with 324 reports from 1983. The present findings were limited to the type of the diagnostic procedure which in principle, for a lesion suspected of being a melanoma, should have been an excisional biopsy. Punch, incisional and 'shave' biopsies, excochleation (spooning-out), electro-coagulation, cryotherapy, etc., were considered to be inappropriate procedures.

The results showed that 62 patients with a melanoma had been diagnosed by general practitioners, 402 by surgeons, 103 by plastic surgeons, 124 by dermatologists and 16 by those in other disciplines. The description by the pathologists indicated that plastic surgeons in 1986 always performed an excisional biopsy and surgeons performed partial biopsies only in a small percentage of the cases (2%). However, punch biopsies were performed by dermatologists in 39% of the cases, about the same frequency as in 1983 (45%).

The melanomas seen by dermatologists were smaller than those seen by surgeons with a median thickness according to Breslow of 0·72 mm, whereas those seen by surgeons were 1·25 mm thick.

We consider that the dermatologists in the Netherlands are still performing too many punch biopsies on pigmented skin lesions and they have not changed their practice after the consensus conference.

Moles, sunlight and melanoma risk

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The aetiology of melanoma of the skin remains unsettled. Sunlight is often regarded as a major risk factor. Chronic cumulative occupational exposure to ultraviolet (UV) radiation is probably less important than acute intermittent recreational exposure. Sunburn seems to be an especial risk. However, the role of sunlight is limited and other risk factors are also important, e.g. skin phenotype and the number of moles. Environmental chemical carcinogens form a neglected field of research. The situation is rather complicated since the above risk factors (mole proneness, UV radiation, skin colour and xenobiotics) have interrelationships. During the last few years we have studied the relation between: (i) Sunlight and number of moles. Sun behaviour (average periods of sunbathing; frequency of holidays in sunny climates; use of artificial UV sources) showed no correlation with mole counts. This was true irrespective of the sex and skin colour of the individuals. There was also no correlation between the occurrence of dysplastic naevi and the degree of sun exposure. (ii) Skin colour and number of moles. Studying Caucasian children and young adults we found that skin colour was an important denominator of mole proneness: those with a light complexion had the highest mole count. In a small subset of oriental and negroid descent very low numbers of naevi per individuals were observed. (iii) Xenobiotics and number of moles. Mole counts in children treated with multiple cytostatic therapy for childhood cancer were studied. These counts were compared with those of their healthy brothers and sisters and were significantly higher indicating the induction or activation of naevi by cytostatic agents.

Kathon CG allergy: practical information for the dermatologist and the patient

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The preservative Kathon CG contains a mixture of 0·375% methylisothiazolinone and 1·125% methyl(chloro)isothiazolinone and is an important cause of cosmetic allergy.[1] Patch testing with the Kathon CG-containing products themselves usually yields false-negative results, so it has been advised to add Kathon CG 100 ppm in water to the European standard series.[2] Members of the Dutch Contact Dermatitis Group have tested 3472 patients suspected to have allergic contact dermatitis with Kathon CG and 163 (4·7%) gave a positive reaction. In 70% of these patients, the reaction to Kathon CG was relevant to their dermatitis and nearly all the cases had been caused by leave-on products, especially moisturising preparations.

It is difficult to advise patients allergic to Kathon CG on the future use of cosmetics since

TABLE 1. Cosmetic products which usually do NOT contain Kathon CG

Aftershave lotion	Eye shadow (powder)
Bath oil (not foaming)	Eyebrow pencil
Bath salt	Perfume/eau de toilette
Deodorant lotion/stick	Permanent wave fluid
Hair bleach	Powder (face, body, feet)
Hair spray	Soap bars
Lipstick/pommade	Suntan oil
Nail lacquer (remover)	

TABLE 2. Brands of cosmetic products that do NOT contain methyl(chloro)isothiazolinone (Kathon CG)*

4711	Lagerfeld
Brylcream	Mandate
Christian Dior	Miss Sporty
Clinique	Old Spice
Derringer	Roc
Desert Flower	Schwarzkopf
Elisabeth Arden	Shisheido
Helena Rubinstein	Theatre
Dr. van de Hoog	

* In The Netherlands.

there is little information on the ingredients contained in these preparations. Several categories of cosmetics though, may be used without problems (Table 1).

To obtain more information, members of the Dutch Cosmetic Association were asked to provide a list of all products their company imports and/or manufactures, and to identify which of these contained methyl(chloro)isothiazolinone. Several stated that they did not use Kathon CG in any of their products (Table 2). Others have recently decided to omit the preservative from all their cosmetic products.

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The cryosurgical open-cone-spray method: a proposal to standardization

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Cryosurgery of skin malignancies, especially of the basal cell carcinoma is generally performed

by the open-cone-spray method as introduced by Torre in 1977.[1,2] The supply of liquid nitrogen, normally used as cryogen, is influenced by several different variables, such as the spray pattern, the spraytip diameter and the distance between spraytip and target area.

In this study a gelatin model was used for the freezing experiments. Cone diameters 11, 16 and 21 mm are most widely used in dermatological practice and were used in this study. The temperatures of the advancing freeze front were recorded by placing thermocouples are various depths in the centre and at the end of the cones. For each cone the variables were compared with each other by statistical and graphical analysis.

Our preliminary results indicate that a standardized procedure can be proposed as follows:

(i) By comparing different spray patterns, i.e central, circular and 'paintbrush' spraying, the central spray pattern showed the fastest extension of the freeze front. This implied the introduction of a modified cone with the spraytip fixed in its centre.

(ii) The spraytip diameters 0·6, 0·8, 1·0 and 1·7 mm were compared with each other. The spraytip diameter 0·8 mm provided the best results for all cone diameters (11, 16 and 21 mm), especially with regard to the temperature decline at the edge of the cone. The spraytip diameters 1·0 and 1·7 mm gave equal results to the spraytip diameter 0·8 mm, but nevertheless they caused an evident run-off of liquid nitrogen. The spraytip diameter 0·6 mm is insufficient for at least the cone diameters 16 and 21 mm.

(iii) Using the spraytip diameter 0·8 mm, an optimal decline of temperature is achieved from a distance of 15 mm to the surface of the target area. In this experiment the distances 5, 10, 15 and 20 mm were compared with each other.

The experiments indicate that a central spray pattern, using a spraytip diameter of 0·8 mm, fixed in the center of the cone from a distance of 15 mm to the surface of the target area, gives an optimal supply of liquid nitrogen. This preliminary report proposes a standardized procedure and a modification of Torre's open-cone-spray method for the cone diameters 11, 16 and 21 mm.

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Abnormalities of soft tissue and bone associated with melanosis naeviformis of Becker—a clinicoradiological study in 40 patients

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In 1983 Glinick *et al.* drew attention to the possible association of melanosis naeviformis of Becker (MNB) with a variety of isolated or combined developmental defects of soft tissue and bone.[1] These structural anomalies have been described in a total of 16 MNB-patients and tend

TABLE 1. Summary of reported structural abnormalities in MNB-patients

	Abnormality	Sex	Reference*	Year of publication
1	Hypoplasia ipsilateral breast	F	Copeman and Wilson Jones	1965
2	Shortening ipsilateral arm	F	Copeman and Wilson Jones	1965
3(a)	Hypoplasia ipsilateral breast	F	Mascaró *et al.*	1970
(b)	Thoracic scoliosis			
4	Spina bifida L5	M	Naranjo *et al.*	1980
5	Severe kyphoscoliosis	M	Tymen	1980
6	Scoliosis	M	Tymen	1980
7	Enlarged ipsilateral foot	F	Lucky *et al.*	1981
8(a)	Hypoplasia ipsilateral breast	F	Glinick *et al.*	1983
(b)	Hypoplasia ipsilateral arm			
(c)	Lumbar spina bifida occulta			
9(a)	Pectus carinatum	M	Glinick *et al.*	1983
(b)	Spina bifida occulta L5			
10	Thoracic torsion scoliosis	F	Smolle	1983
11(a)	Hypoplasia ipsilateral breast	F	Moore and Schosser	1985
(b)	Aplasia ipsilateral M. pectoralis major scoliosis			
(c)	Slight lumbar scoliosis			
12	Pectus carinatum	M	Glinick and Alper	1986
13	Pectus carinatum	F	Glinick and Alper	1986
14	Hypoplasia ipsilateral arm + hand	M	Glinick and Alper	1986
15	Accessory scrotum	M	Szylit *et al.*	1986
16	Hypoplasia ipsilateral breast	F	Blanc *et al.*	1988

* References available on request to Dr. R-F.H.J.Hulsmans.

to be mostly hypoplastic in nature and ipsilaterally localized (Table 1). Since MNB occurs more frequently in men (M:F-ratio = 4–5:1), it is surprising that structural anomalies were reported more often in females (M:F = 7:9).

We randomly selected 40 MNB-patients and thorough physical and radiological examinations of the vertebral columns were performed in all these subjects. Thirty-three male and seven female patients were included in this study. The M:F-ratio in our group corresponded with the estimated M:F-ratio of MNB in the normal population. The age of the patients ranged from 2–47 years (mean, 24). Physical examination revealed no gross asymmetry of the trunk, extremities and breasts, apart from a scoliosis in our youngest patient.

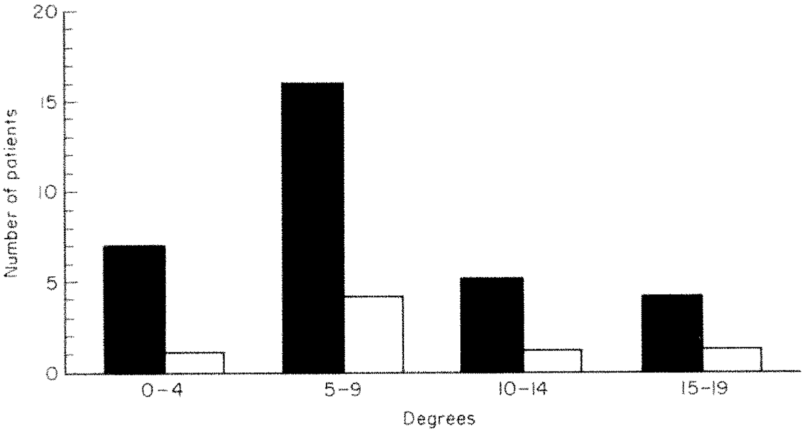

FIGURE 1. Cobb angles in patients with MNB ($n = 39$). ■, Males; □, Females.

The thoracic and lumbar curves were measured independently by two radiologists according to the method of Cobb. The Scoliosis Research Society defines scoliosis to be present when spinal curvatures show Cobb angles $\geqslant 10$. In our MNB-group nine out of 32 men (28·2%) and two out of seven women (28·5%) appeared to have scoliosis (Fig. 1). Our youngest patient was excluded from this part of the study because he clearly showed a secondary non-idiopathic congenital scoliosis.

The mean incidence of idiopathic scoliosis (IS) in the normal population is approximately 2–3% with a M:F-ratio of 1:2–3.[2] The frequency of scoliosis in our MNB-group appeared to be 10 times as high and differed significantly from the incidence of IS ($P < 0{\cdot}05$).

Moreover, the M:F-ratio of our scoliotic MNB-patients (4–5:1) corresponded well with the estimated M:F-ratio of MNB in the normal population and inverted significantly ($P < 0{\cdot}05$) when compared with the M:F-ratio of IS (1:2–3). We did not succeed in establishing a correlation between the localization of MNB and the height or direction of the scoliotic curvatures. Furthermore, we observed a variety of congenital anomalies of the vertebral column and ribs, which in all but one case could not be held responsible for an eventually coexistent scoliosis. These anomalies consisted of lumbalization of S1 (4 cases), spina bifida of L5 of S1 (4 cases), sacralisation of L5, an accessory pair of ribs (2 cases), spondylolysis (1 case) and hemisacralisation of L5 (1 case). The frequency of these anomalies in the normal population is high[2] and the incidence in our MNB-group did not allow any statistical evaluation. It is unclear and even doubtful whether the vertebral body anomalies as observed in our study and reported in the literature represent true associations with MNB. Larger groups of MNB-patients are required. However, in our youngest patient with hemivertebrae, fused ribs and an accessory cervical rib we consider there is an association, since most of his congenital anomalies responsible for his scoliosis were localized in the metamere of his MNB.

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CASE REPORTS

Naevoid basal cell carcinoma syndrome with concomittant bilateral segmental neurofibromatosis

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History. A 31-year-old male has had since childhood flesh-coloured lesions in a circumscribed area on his abdomen and both flanks. During the last 5 years an increasing number of erythematous and pigmented skin tumours have developed on his face and trunk. Recently he had been treated for jaw cysts. The family history was negative for signs and symptoms of naevoid basal cell carcinoma syndrome and neurofibromatosis.

Examination. In the dermatome of T8 there were multiple small flesh-coloured nodules (Fig. 1). There were no depigmented or café-au-lait macules. On the face (Fig. 2) and trunk multiple, partly flesh-coloured, partly erythematous and hyperpigmented, glassy papules and nodules with telangiectasia were seen which varied in diameter from 3 to 20 mm. The palms of the hands showed typical Calnan pits. The head circumference measured 62·5 cm and there was frontal bossing, broadening of the nasal bridge and small dental elements were noted. Ophthalmological examination revealed hypertelorism and a divergent squint. Lisch nodules, colobomata, cataract and glaucoma were absent.

Radiology. Calcification of the falx cerebri was noted on the skull X-ray. A dental X-ray showed a remnant of a mandibular jaw cyst. Spina bifida occulta of L5 was found.

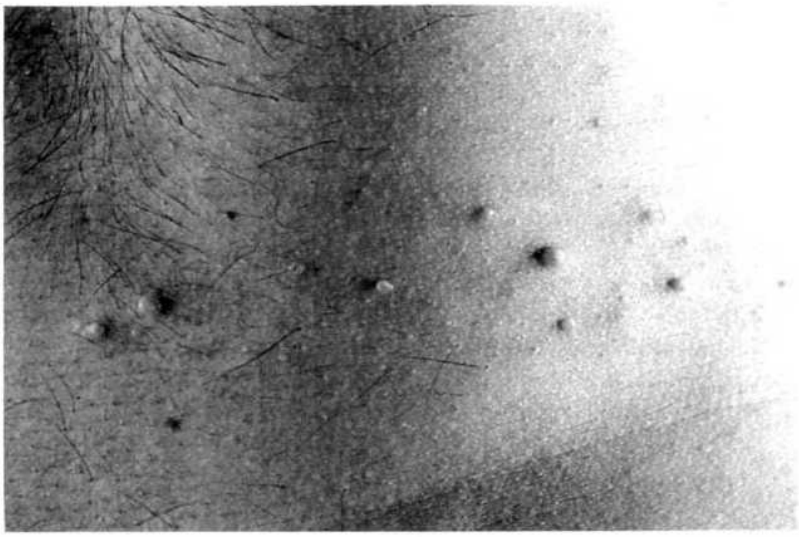

FIGURE 1. Neurofibromas in the T8 dermatome.

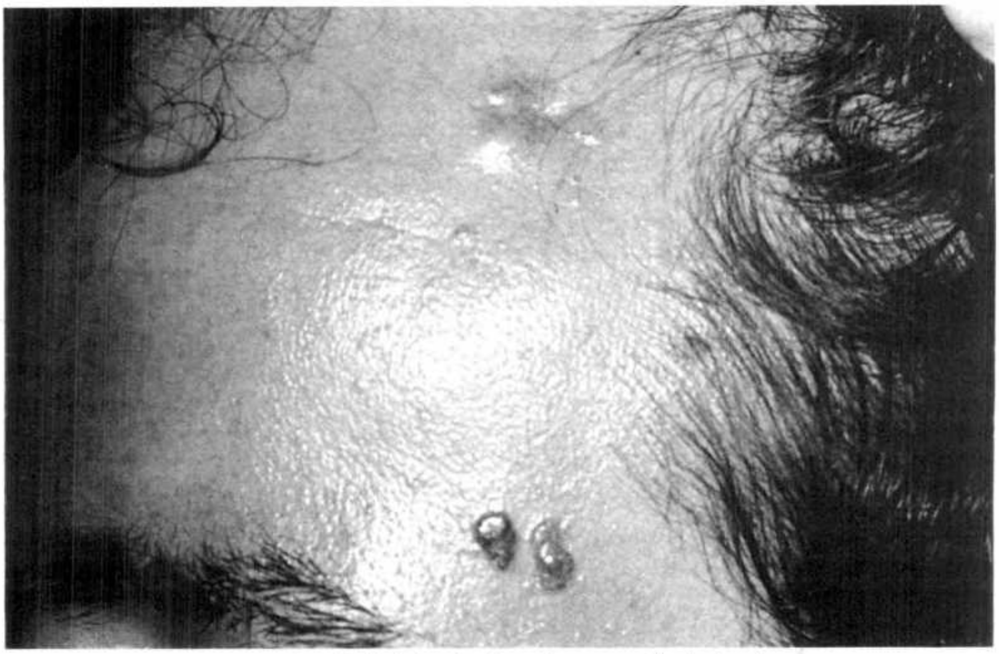

FIGURE 2. Multiple basal cell carcinomas on the forehead.

A CAT scan confirmed the calcification of the falx and revealed the presence of a hydrocephalus with calcification of the chloroid plexus.

Histology. Multiple biopsies of lesions from the T8 dermatome showed a proliferation of spindle shaped cells in the dermis with hyperchromatic nuclei and a slight eosinophilic cytoplasm with positive staining with S-100 consistent with the diagnosis of neurofibromas. The multiple facial and truncal tumours showed the histology of solid and superficial basal cell carcinomas.

Treatment. The basal cell tumours were treated with various techniques that included surgical excision, cryosurgery and electrodesiccation.

Discussion. The naevoid basal cell carcinoma syndrome has an autosomal dominant inheritance with a wide variety of clinical manifestations.[1] Segmental neurofibromatosis is characterized by segmentally arranged café-au-lait spots and/or multiple neurofibromas that may be localized in a uni- or bilateral fashion and is suggested to develop from a spontaneous genetic mutation.[2,3]

This case describes the coexistence of naevoid basal cell carcinoma syndrome with bilateral segmental neurofibromatosis.

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Dowling-Degos disease associated with profuse lentigines

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History. A 47-year-old woman noticed for at least a year, progressive pigmentation in the anogenital region. The lesions were sometimes itchy. Her 44-year-old sister had identical skin lesions in the same region with itching during and following menstruation.

Examination. The patient and her sister showed symmetrical, reticulated, hyperpigmented macules involving the neck, axillae, labia majora, perineum and intergluteal areas (Fig. 1). There were multiple lentigines scattered on arms, legs and trunk (Fig. 2). Erythrosis, pitted scars or comedo-like lesions were absent. The older sister had a nodule in the left preauricular region.

Histology. Biopsy specimens from the genital region of both sisters showed areas of thinning of the epidermis, with branched epithelial downgrowths associated with intraepidermal keratin cysts. The tips of the downgrowths were hyperpigmented.

The histology of the preauricular nodule showed a follicular cyst with digitate projections of epithelium surrounded by a chronic inflammatory and granulomatous infiltrate. The biopsy of a lentiginous macule of the upper arm showed the histology of a lentigo simplex.

Discussion. Dowling-Degos disease (DDD) is a rare, benign, autosomal-dominant pigmentary disorder,[1] characterized by punctate, confluent, hyperpigmented macules of the flexures and pigmented perioral pits. Epidermoid cysts, abcesses and pilonidal sinus have been described in DDD.[1,2] Since DDD shows considerable overlapping of symptoms with Haber's syndrome both DDD and Haber's syndrome have been suggested to be variants of a complex

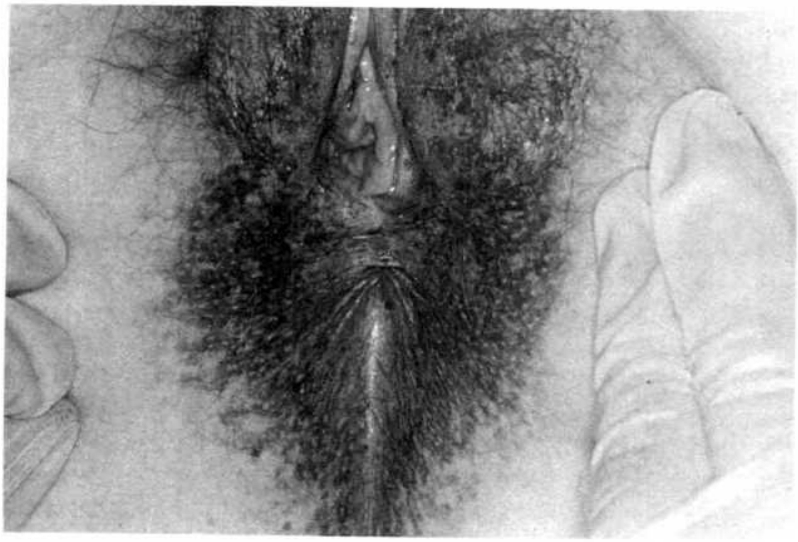

FIGURE 1. Genital and perianal reticulate hyperpigmentations.

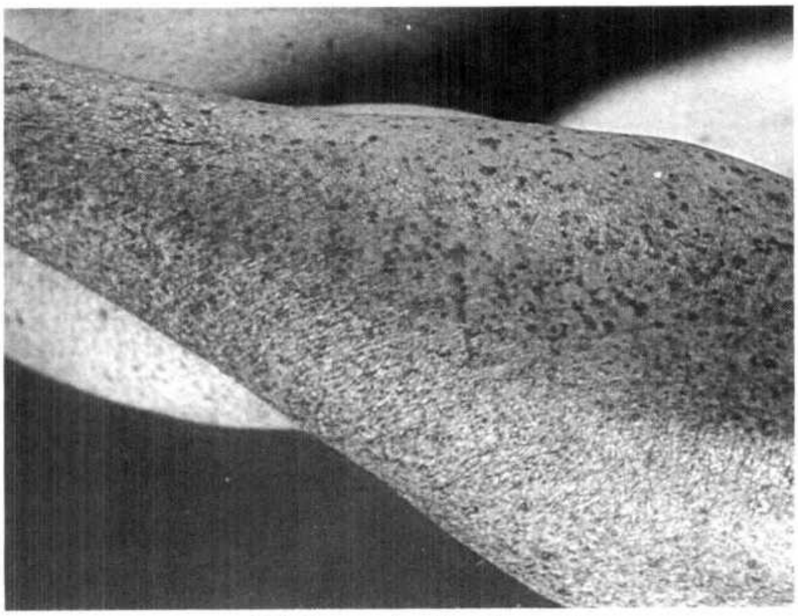

FIGURE 2. UV-photography of lentigines on forearm.

genodermatosis with a broad spectrum that may involve other genodermatoses sharing the features of reticulate hyperpigmentation and/or cysts.[3,4] To our knowledge, an association with multiple lentigines scattered profusely over the body, has not been previously reported.

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Bowenoid papulosis and invasive squamous cell carcinoma of the vulva in a virgo intacta

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History. A 32-year-old woman had a 2-year history of genital warts, which progressed in size and number and were associated with pruritus and pain. In the years preceding her genital complaints she has had common warts on her hands. Except for one homosexual contact she denied any sexual relationship.

Examination. The left labium minus was white with keratotic and hyperpigmented papules and plaques. Beneath the clitoris an eroded area was present that extended to the intact hymen.

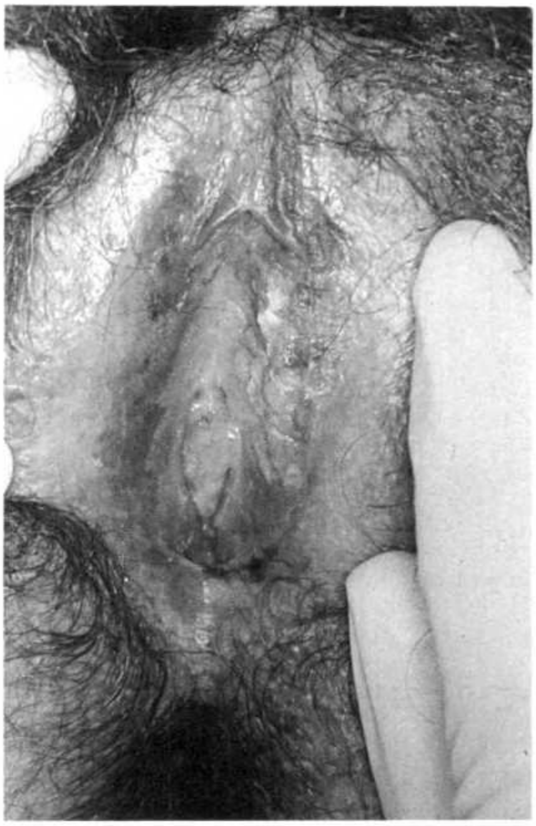

FIGURE 1. Verrucous, hyperpigmented papules and plaques on the left labium minus and pigmentation on the commissura posterior.

On the right labium majus and the posterior commissure there were dark pigmented macules (Fig. 1). In the perianal region similar pigmentation and verrucous lesions were noted. There was no inguinal lymphadenopathy.

Histology. Left labium minus. The epidermis showed papillomatosis with elongation of the rete ridges. There were dyskeratotic keratinocytes with polychromatic and polymorphous nuclei, and many mitoses were seen. The posterior commissure had a normal epidermis with hyperpigmentation of the basal layer. In the anterior commissure an area of Bowenoid papulosis, invading squamous cells with varying atypia and individual keratinization were noted. Collagen type IV staining revealed disruption of the basal membrane by the tumour cells. In the papular verrucous as well as in the macular hyperpigmented lesions human papilloma virus (HPV) common antigen was demonstrated in several keratinocytes. However, *in situ* DNA hybridization was negative for HPV-types 2, 4, 5, 6, 8, 11, 16 and 18.

Treatment. A total vulvectomy with removal of the inguinal lymph nodes was performed. The margins of the excised vulvar tissue was free of bowenoid papulosis and squamous cell carcinoma and there was no evidence of any metastasis to the regional lymph nodes.

Discussion. Bowenoid papulosis of the genitalia (BPG) most frequently occurs in young, sexually active adults. Various types of HPV are thought to be involved in the pathogenesis of BPG.[1-3] In our patient, the transmission and type of the causative HPV remains enigmatic, though a history of viral warts on other parts of the body in BPG patients has been described.

In general, BPG is considered to be a benign disease with a tendency to spontaneous regression. However, in more recent literature coexistence with or transformation to invasive carcinoma is recorded.[4,5] These data and our case demonstrate the need for a more careful approach towards BPG.

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Hereditary persistent naevus medullaris frontalis et occipitalis (Unna) with classical and ectopic localizations

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History. A 31-year-old woman and her two daughters aged 1 and 3 years had had since birth persistent erythematous macules on the face, neck and back. None of them suffered from any neurological and/or orthopaedic condition.

Examination. The mother showed two moderately circumscribed erythematous macules on the forehead and on the neck. A group of small circumscribed erythematous macules was observed in the paravertebral region of the thoracolumbar and lumbosacral part of the back (Fig. 1). The long axis of these dorsal lesions followed the skin cleavage lines.

The eldest daughter showed an erythematous macule on the forehead and on the neck. A small number of grouped erythematous macules was seen on the back in the left paravertebral part of the thoracic spine and in the lumbosacral paravertebral regions (Fig. 2). The long axis of the dorsal lesions also followed the skin cleavage lines.

The youngest daughter showed circumscribed erythematous macules on the forehead, on both upper eyelids and on the neck. An aggregate of erythematous macules was present on the lumbosacral part of the back.

Discussion. The Unna naevus is also known as naevus vasculosus nuchae, angel's kiss and stork bite, nape naevus, erythema nuchae, salmon patches and naevus flammeus nuchae. In our opinion, van der Werf proposed the most appropriate term: 'naevus medullaris frontalis et occipitalis (et sacralis)'.[1] They may very frequently be observed in newborn infants. Usually

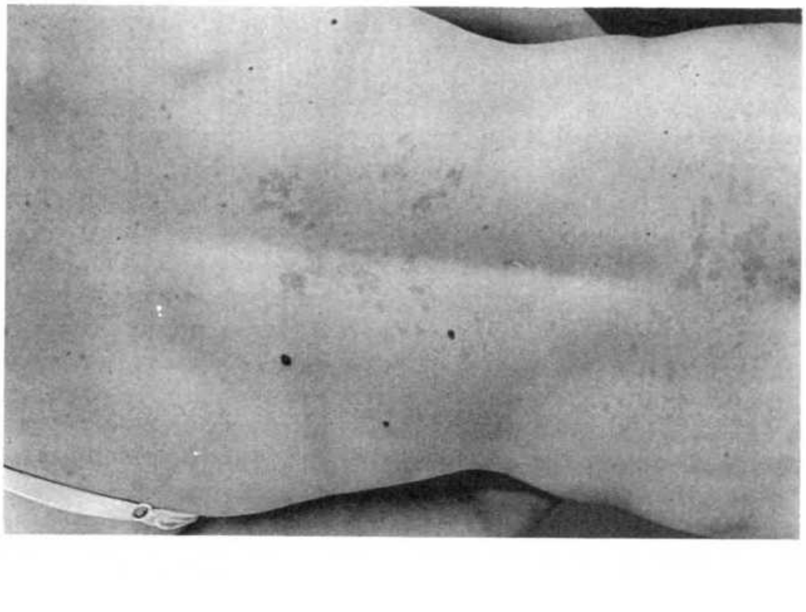

FIGURE 1. Thoracolumbar and lumbosacral naevi in the mother.

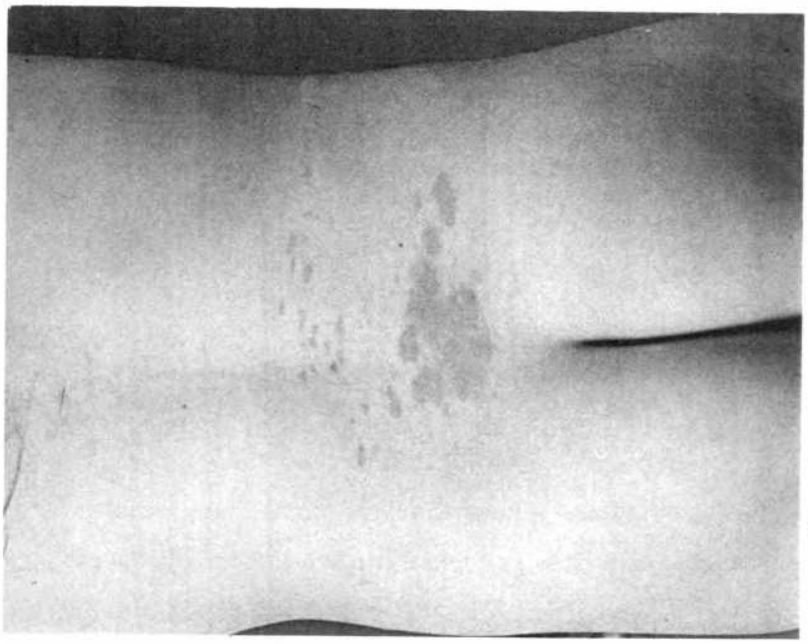

FIGURE 2. Thoracic and lumbosacral naevi in the eldest daughter.

they are symmetrically localized around the midline on the root of the nose and the glabella, upper eyelids and on the neck. Oster and Nielsen also observed interscapular telangiectases in a large number of schoolchildren.[2] Fifty per cent of the nuchal naevi vanish before the end of the first year of life. Part of the other half persists up to adolescence or remains present throughout lifetime.[2] Unna naevi may be inherited as an autosomal dominant trait with variability of penetrance and phenotypic expression.[3]

Sacral lesions seem to be rare and have only been described in two brothers by van der Werf.[1] To our knowledge, ectopic thoracolumbar lesions have not been reported previously.

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Angioma lobulatum of Wilson Jones (Acquired 'tufted' angioma)

(This case was also presented at the Journées Dermatologiques de Paris, March 9–12, 1988).

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History. A 31-year-old man rapidly developed over a period of a few months an asymptomatic red patch on his right upper arm (Fig. 1). The lesion then became static and persisted. The histology of a punch and excision biopsy suggested an early Kaposi's sarcoma.

Examination. On the dorsal aspect of the right upper arm extending into the right axilla there was a dull, irregularly circumscribed, slightly elevated and centrally indurated erythematous plaque 8 cm in diameter. On diascopy of the lesion with a glass microscope slide the erythema disappeared but left a yellowish haematoma-like colour.

Laboratory tests. The white cell count was normal though there was a slight increase in eosinophils. HIV-antigen and -antibodies were negative. Seldinger angiography of the right subclavial artery failed to show AV-fistulae and filling of the skin lesion with contrast material. X-rays of the thorax and right shoulder revealed no abnormalities.

Histology. At various levels in the dermis closely packed capillary and normotypic endothelial proliferations with a 'cannon ball' configuration were seen (Fig. 2). In some instances these were surrounded by semilunar dilated capillaries. A few spindle cells and a discrete infiltrate consisting of lymphocytes, histiocytes and some eosinophils with focal erythrocyte extravasation and siderophages were noted. Using a panel of enzymehistochemical and immunohistochemical markers for vascular endothelium and vascular wall constituents (alkaline phosphatase,

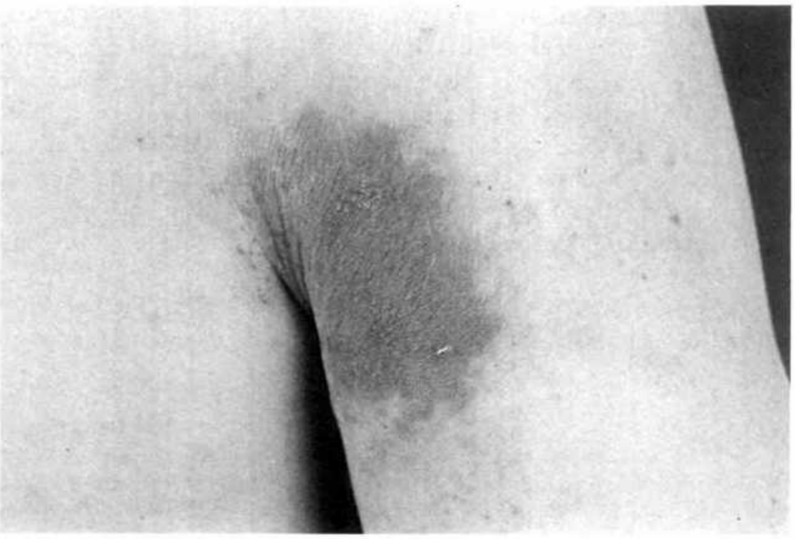

FIGURE 1. Slightly elevated, indurated erythematous plaque on the right upper arm.

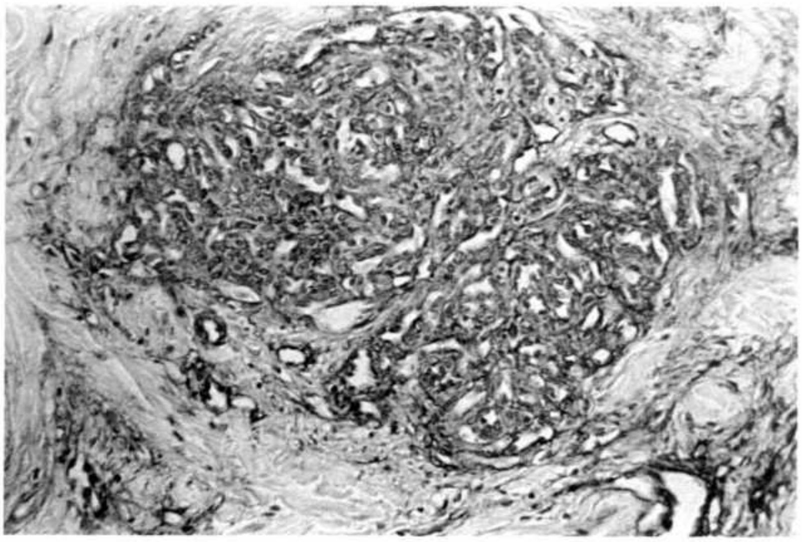

FIGURE 2. Collagen type IV staining: 'cannon ball'-like proliferation of dermal capillaries and endothelium.

adenosine triphosphatase, factor VIII-related antigen, lectin UEA-I, EN-4, PAL-E, HLA-DR, collagen type IV, laminin, D66) performed on snap-frozen material of this tumour and six AIDS-related Kaposi's sarcomas, we did not succeed in finding clear differences in enzyme activity, lectin binding and antigen expression.

Discussion. Our case demonstrates the difficulty in diagnosis of an acquired angiomatous condition when AIDS related Kaposi's sarcoma now occurs. During his Dowling oration about malignant vascular tumours in 1976 Wilson Jones briefly discussed the clinical and histological features of a benign vascular tumour seen by him in 10 patients which he described as 'acquired tufted angioma' (ATA).[1] Several reports have confirmed this distinct clinicopathological entity.[2,3] The scalp, neck, upper part of the trunk and proximal extremities are common sites for ATA and the clinical picture may vary from a single to various nodules or plaques. Annular forms[4] or lesions with peripheral satellites[3] may occur. Histologically Kaposi's sarcoma differs from ATA by the diffuse pattern of the dermal vascular proliferation, the abundance of spindle cells and the presence of some atypia of endothelial cells. In ATA these are fairly well circumscribed vascular proliferations, with paucity of 'spindling' and the endothelial cells appear normal.

We propose the term 'angioma lobulatum of Wilson Jones' for ATA to credit its delineator and to distinguish it from an entity called lobular capillary hemangioma that might be related.[1]

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Angiolymphoid hyperplasia with eosinophilia

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History. A 53-year-old woman presented with a 10-year history of premenstral itching localized to the anus and vulva. In the last 3 years the localized and intermittent itching became persistent and generalized. In 1986 an itchy anal skin tag was excised but not histologically examined. Our patient had no intestinal symptoms.

Examination. In the perianal area, at a 9 and 10 o'clock position there were two flat, swollen, slightly indurated erythematous nodules which were partly eroded and crusted. At a 12, 3 and 6 o'clock position several soft anal skin tags were present. The right labium majus showed multiple, small, smooth and erythematous nodules (Fig. 1). There was no inguinal lymphadenopathy.

Laboratory test. Routine laboratory examinations were all within normal limits including the total eosinophil count. Serum IgE was moderately raised with a value of 210 kU/l ($n \leqslant 50$ kU/l) and no specific IgE against *Candida albicans* was detected.

Histology. Apart from a reactive acanthosis of the epidermis and elongation of the rete ridges the anal and vulvar nodules showed a dermal proliferation of thick- and thin-walled blood vessels with prominent and sometimes vacuolated endothelium focally obliterating the lumen. Fig. 2.

A perivascular and diffuse inflammatory infiltrate composed of lymphocytes, histiocytes, eosinophils and a few plasma cells was observed throughout the dermis without aggregation into lymphoid follicles.

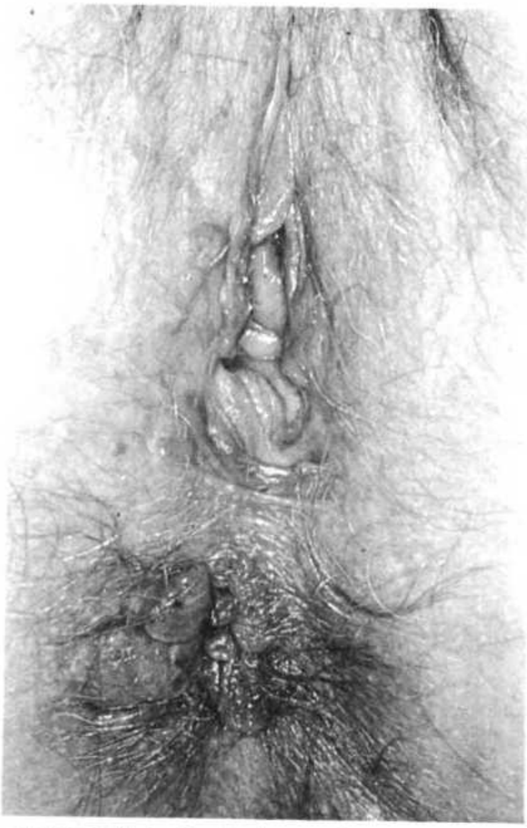

FIGURE 1. Perianal and vulvar erythematous nodules.

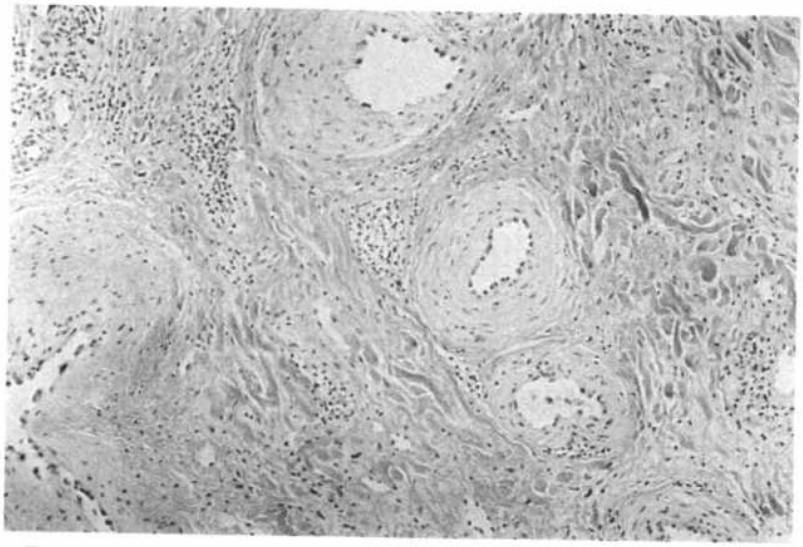

FIGURE 2. Thick-walled vessels with prominent endothelial cells with the aspect of high endothelial venules.

Treatment. The vulvar and anal lesions were removed in several sessions and during this period the serum IgE lowered to 118 kU/l and returned to normal values following treatment. The localized and generalized pruritus ceased after removal of the last lesion.

Discussion. Angiolymphoid hyperplasia with eosinophilia is considered to be an acquired benign vascular anomaly with a predilection for the head and neck region. The clinical lesions appear as single or multiple red-brown papules or as subcutaneous nodules characterized histologically by an anomalous vascular proliferation and inflammation at different levels of the dermis and/or subcutaneous tissue.

Until 1969 Kimura's disease was almost exclusively reported in the Japanese literature.[1] In that year Wells and Whimster reported nine cases under the name of angiolymphoid hyperplasia with eosinophilia (ALHE).[2] At present there is no consensus as to whether Kimura's disease and ALHE represent the same condition.[3,4]

The localization of ALHE in our patient is unusual and we have not found a similar case in the literature. Our patient suffered from generalized pruritus which is rarely reported in localized ALHE. This symptom ceased during therapy with a fall in the serum IgE level.[5]

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Muir–Torre syndrome

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History. A 63-year-old woman presented with multiple asymptomatic papules on her face. At the age of 41 our patient had a hysterectomy because of uterine fibroids. Histological examination of the uterus showed endometrial hyperplasia with focal atypia and benign fibroids. In the family history there was a remarkable number of members with an early onset of cancer that was primarily localized to the colon and rectum (Fig. 1).

Examination. Multiple flesh-coloured papules on the face.

Histology. Four biopsies showed the picture of a sebaceous hyperplasia and another five revealed sebaceous adenomata.

Laboratory tests. ESR, RBC, WBC and diff., CEA, Ca 125, liver- and kidney functions were all normal. Urinalysis showed normal protein and sediment results. Faecal tests were all

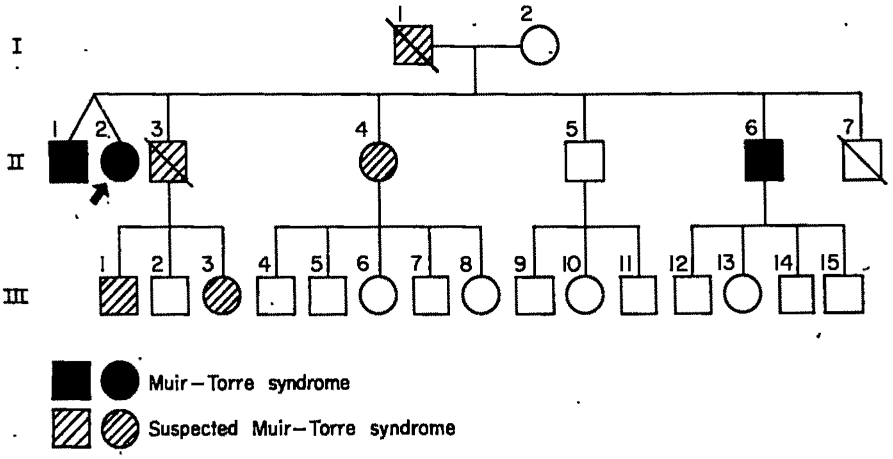

FIGURE 1. Family history.

I 1 Adenocarcinoma of the colon (deceased at age 67).
II 1 Adenocarcinoma of the rectum (age 34).
Adenocarcinoma of the colon (age 43).
Adenocarcinoma of the colon (age 56).
2 Uterine fibroids, endometrial hyperplasia and -atypia (age 41).
Facial sebaceous hyperplasia and adenomas.
3 Metastatic melanoma (died age 31).
4 Solid basal cell carcinomas on the back and the face.
6 Adenocarcinoma of the rectum (age 34).
Adenocarcinoma of the colon (age 52).
Facial sebaceous hyperplasia and adenomas.
7 Died at age 2 months. Intestinal pathology.
III 1 Facial sebaceous hyperplasia.
3 Cervical dysplasia PAP IV (age 29).

negativeand a total colonoscopy showed no abnormality. Echograms of the kidneys and ovaries revealed no abnormality.

Discussion. The Muir–Torre syndrome is characterized by multiple early onset low-grade malignancies in association with a spectrum of sebaceous neoplasms, namely sebaceous hyperplasias, adenomas and carcinomas, and possibly also keratoacanthomas. The syndrome is a genetic condition, probably transmitted as an autosomal dominant with variability in phenotypic expression and is considered to be a variant of the Family Cancer syndrome.[1–4] The visceral malignancies predominantly involve the genitourinary system and gastrointestinal tract, particularly the colon and rectum. Colonic polyps are frequently found.[2,4] Other cutaneous carcinomas (basaliomas, spinaliomas[3–5] and melanoma[3,4]), extracutaneous carcinomas and lymphoproliferative malignancies[2,4] have also been described. In our family two basaliomas and one melanoma were observed. It is difficult to be certain whether these are coincidental or not. The sebaceous neoplasms may precede or coincide with the visceral carcinomas but most often they do not manifest until some 10 years later. Their value as a cutaneous marker of internal malignancy for the Muir–Torre patient is therefore limited.[5] Once the diagnosis of Muir–Torre syndrome is made, regular check-ups of all family members at risk

TABLE 1. Screening protocol for family members

Screening protocol	Regularity of check ups
Physical examination	yearly
Laboratory tests	yearly
ESR, RBC, WBC and diff.	
CEA, Ca 125	
Urinalysis: protein, sediment	
Faecal occult-blood test	
Cervical smear	yearly
Echogram of ovaries	yearly
Echogram of kidneys	2 years
Colonoscopy	5 years
(positive for polyposis)	3 years

are mandatory to detect internal malignancies.[6] A review of the literature and a multidisciplinary approach in the study of our family have led to the development of a screening protocol for all family members from the age of 20 onwards (Table 1).

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British Journal of Dermatology (1989) **121**, 541–544.

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Linear IgA dermatosis with severe arthralgia

SIR, I wish to add a further case of linear IgA dermatosis associated with severe arthralgia to those recently described.[1]

A 25-year-old woman presented with an 8-week-history of sudden onset arthralgia affecting her knees, wrists, elbows and shoulders. Treatment with ibuprofen was followed 1 week later by the development of a non-itchy eruption starting on the upper arms and spreading rapidly to involve the face. The cutaneous signs consisted of multiple small (< 1 cm in diameter) flaccid bullae with no erythema or other changes either on the underlying or surrounding skin (Fig. 1). There was no mucosal involvement. The non-steroidal anti-inflammatory drug (NSAID) was changed to indomethacin and then to naproxen. No improvement was seen in the cutaneous manifestations but there was some improvement of her joint symptoms.

The following investigations were negative or normal: full blood count, serum estimations of urea and electrolytes, creatinine, calcium, phosphate, total protein and protein electrophoresis, albumin, liver function tests, urate; bacterial and viral cultures of blister fluid, mid-stream specimen of urine, paired serology for the following viruses, herpes simplex, varicella-zoster, rubella, coxsackie B 1-5 and cytomegalovirus, serum anti-nuclear factor, rheumatoid factor, complement levels, anti-Ro antibody, anti-La antibody and X-ray of hands. Serum C-reactive protein was elevated at 38 mg/l (normal < 10 mg/l) and serum IgG marginally elevated at 19·4 g/l (7–19 g/l). Serum IgA and IgM were normal.

A biopsy of one of the cutaneous lesions showed a subepidermal bulla and direct immunofluorescence of

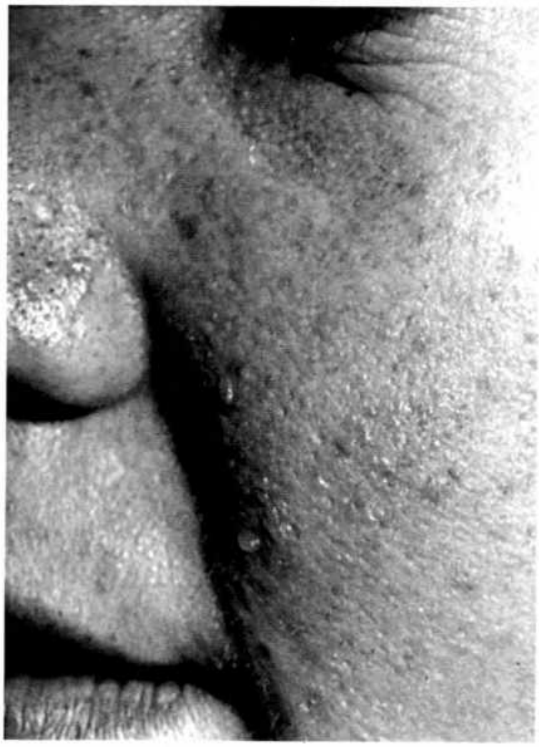

FIGURE 1. Translucent bullae on the face. Note the normal appearance of underlying and surrounding skin.

perilesional skin revealed a linear band of IgA and C3 at the dermo-epidermal junction. A diagnosis of linear IgA dermatosis was made.

Treatment with dapsone 100 mg daily resulted in rapid resolution of the rash. The dose was later reduced to 50 mg daily without recurrence. The patient's joint symptoms however, did not resolve although they improved relative to the period before treatment with dapsone. Six months following diagnosis the patient developed a mild phototoxic reaction (kindly confirmed by Dr. J. Ferguson, Photobiology Unit, Ninewells Hospital, Dundee, U.K.) which was thought to be due to naproxen.

Sixteen months after the initial diagnosis the cutaneous eruption is well controlled with dapsone 50 mg daily. One attempt to stop the treatment with this drug resulted in a recurrence. The patient still suffers from arthralgia but this is helped by the intermittent use of NSAIDs.

As the more common causes of arthralgia have been excluded in this case by investigations, and subsequent clinical course, this patient contributes further evidence in favour of an association between linear IgA dermatosis and arthralgia. It is also interesting to note the association between the onset of the cutaneous eruption and the preceeding use of NSAIDs — a relationship which has recently been described.[2]

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Venous stasis ulcers, stasis dermatitis and mothers' political ambitions for their offspring

SIR, In their description of chronic leg ulceration with livedoid vasculitis (*Br J Dermatol* 1989; **120:** 101–5) Rustin and colleagues state that their patient had 'no evidence of chronic venous stasis'. But what does this mean?

'Venous stasis' is a term which is commonly applied to the features we associate with venous hypertension of the legs, namely oedema, pigmentation, atrophie blanche etc. but the evidence that these features are due to or even associated with stasis of flow in the veins is very scanty. In 1983 I suggested that 'venous stasis' should be dropped from dermatological parlance on the grounds that the oxygen content in the femoral venous blood of a leg affected by venous hypertension is increased, and the venous blood in such limbs has a faster circulation time than normal, neither of which observations would be compatible with stasis.[1]

I waited eagerly to see who would defend the term. Nobody did, though Reilly and Wood[2] pointed out that ulceration of the hand can occur secondary to a radial arterio-venous fistula, presumably due to venous hypertension. In these cases there is certainly no stasis, though there is pericapillary fibrin deposition. Partsch also agreed that we should drop the term 'stasis', since his work with a laser Doppler had shown that in the skin surrounding venous leg ulcers the resting flow levels are the same as in hyperaemic normal skin.[3]

I assumed that people were convinced, but Rosen, talking about the 'quirks of British nomenclature', stated that Americans still use the term 'stasis dermatitis'.[4] I challenged him to explain how stasis could be present in view of the above observations.[5] He replied to the effect that when he was in London he had been called 'Governor' and his mother had always wanted him to be a politician.[6]

Since then the term has continued to be widely used in dermatological journals. Can any users now defend the term, or are all your mothers equally ambitious?

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Low-dose recombinant interferon-α in the treatment of cutaneous T-cell lymphomas

Sir, Several studies have demonstrated that high doses of interferon-α ($30–50 \times 10^6$ IU per day) are effective in the treatment of cutaneous T-cell lymphomas (CTCL).[1–5] However, these high doses induce severe side-effects, and it has been shown that low doses can result in more effective natural killer cell activation than higher doses of interferon-α.[6]

We have treated five patients with CTCL (three stage IIb and two stage IVb) with low doses of interferon-α 2 (Roche) using $3–6 \times 10^6$ IU three times a week. The effects on the Langerhans cells, keratinocytes and the dermal infiltrate in skin biopsies from our patients were studied. Biopsies were taken before treatment and at 2-monthly intervals over a period of 6 months, and from the same region. The biopsies were snap frozen and using an indirect immunological technique were stained with various monoclonal antibodies.

After 8 months of treatment two of the patients, both stage IIb, were in complete remission. Two of the patients, one stage IIb and one stage IVb, were partially improved with a reduction of their cutaneous lesions and the size of their enlarged lymph nodes. One of the patients (stage IVb) was not improved. There were no severe side-effects during the course of treatment.

The immunological studies showed that the Langerhans cells were present in the epidermis even after 6 months of treatment. There was an increase in the number of $CD8^+$ cells, and this was associated with a decrease in the expressivity of CD3 and CD5 antigens on CD4 tumoral cells. This was noted in the patients who had a good clinical response. The percentage of NK cells in the dermis in our patients was low (1–6%). It was also noted that HLA-DR expression was induced or increased on the keratinocytes.

This study confirms that low doses of interferon-α are effective in the treatment of CTCL.[7,8] These low doses produced an improvement in most of our patients, and none of them had any significant side-effects from the administration of interferon-α. Interferon-α does not act by removing Langerhans cells, as occurs with oral psoralen photochemotherapy, and these cells were found in all the biopsies taken from our patients after 6 months of treatment and appeared to be in normal numbers. Interferon-α enhances suppressor/cytotoxic activity and modulates the expressivity of CD3 and CD5 antigens closely related to the T-cell receptor.[9,10] The expression of HLA-DR and Leu 11 in the epidermis following treatment with interferon-α was observed in our patients, and is probably due to a modulation in the secretion of the cytokines IL-1, IL-3 and IL-6.

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British Journal of Dermatology (1989) **121**, 545–549.

Book Reviews

The Dermal Lymphatic Capillaries. JUDIT DAROCZY (1988). Berlin: Springer-Verlag. Pp. 157, 174 figures. ISBN 3-540-19029-5. Price £55.00.

As pointed out in the preface to this volume, although it has been recognized for many years that the lymphatic system of man plays an important part in normal physiology and the pathophysiology of various diseases, there has been relatively little detailed work published on the anatomy and physiology of the lymphatics of the skin. Dr Daroczy has attempted to correct this anomaly, at least in part, by publishing this monograph on the structure and function of dermal lymphatics both in healthy and in certain pathological conditions. This has been achieved by what appear to be careful studies of skin biopsies from various sites in both human and animal material. The majority of the book is concerned with the detailed anatomy of the various parts of the cutaneous lymphatic network and includes comments and illustrations of pericapillary connective tissue, endothelial cells and the valve systems. The bulk of the monograph consists of electron photomicrographs of excellent quality and in many ways is better regarded as an annotated atlas of the ultrastructure of dermal lymphatics than a textbook. In addition to a detailed discussion of the anatomy there are chapters on aspects of the physiology of the lymphatic system and a discussion of the structure and function of lymphatics in various disease states. The choice of the disease states is in some areas slightly idiosyncratic and ranges from syphilis to mucormycosis. Certain tumours where lymphatic proliferation is felt to play an important part, such as Kaposi's sarcoma, are also discussed and most of the references in these sections are fairly up to date. Useful comments are made in various sections of the book on the technical aspects of the demonstration of lymphatics in the skin. These include references to newer monoclonal antibody techniques, such as the use of the PAL-E antibody, originally reported by Schlingemann.

Dr Daroczy has suggested that the information contained in her book should prove useful for investigators and form a basis for further studies. The book has been beautifully produced and is a very attractive volume. Unfortunately, in many areas the English is not as fluent as one would wish and the spelling in some chapters is somewhat variable. Nevertheless Dr Daroczy has produced a most useful book that does indeed fill a gap in the existing literature and any clinician or scientist interested in dermal lymphatics will want to possess a copy for themselves.

N.P.SMITH

Skin Disorders in the Elderly. Edited by B.E.MONK, R.A.C.GRAHAM-BROWN AND I.SARKANY (1988). London: Blackwell Scientific Publications. Pp. 340, illustrations: 60 colour, 98 black and white. ISBN 0-632-02272-8. Price £49.50.

In the preface the editors argue the need for a dermatological book dealing exclusively with skin disorders of the elderly. Having made this point, they state that there have been one or two other books in this field, but these have not concentrated on the problems of the elderly. By implication the editors are only going to discuss the disorders seen in the elderly. They also state that they will cover the scientific basis of ageing in relation to skin diseases, and management and treatment in a better manner than in previous texts. Unfortunately this book does not live up to the editors' intentions.

First, they discuss many conditions which are more likely to be seen in the young and middle-aged rather than the elderly. Three pages are devoted to chicken pox. In the section on bullous diseases, dermatitis herpetiformis and pemphigus are clearly stated not to be diseases of the elderly and yet are discussed at length. The authors of this particular chapter (who are two of the editors) show their lack of experience in treating dermatitis herpetiformis in the elderly by recommending dapsone rather than sulphonamides. The incidence of haemolytic anaemia with dapsone is so high that this is certainly not the first-line drug for use in the elderly. Cardiac or cerebral problems may occur in the elderly with even a small decrease in the haemoglobin concentration.

Although the black and white illustrations are of good quality, many are certainly not of old people.

It is surprising that the editors have chosen not to discuss psoriasis in detail, whilst doing so for rarer skin disorders which occur in all ages, e.g., skin infections. Psoriasis is a common disease and does affect persons in later life, presenting particular problems in the management and treatment in the elderly.

Unfortunately, although the editors know the pitfalls in trying to write a text on skin diseases of the elderly, they have fallen into all three pits. The text is not confined to the diseases of the elderly, their scientific basis section is too brief and not linked to the diseases discussed, and their management and treatment is not confined or directed only to the elderly.

There is no particular reason why this book should be preferred to others in this field.

L.Fry

Atopic Palmoplantar Eczema. Hans Joachim Schwanitz (1988). Berlin: Springer-Verlag. Pp. 144, 52 figures, 16 colour plates. ISBN 3-540-17863-5, SBN 0-387-17863. Price £35.00.

This monograph describes research studies into the pathogenesis of dyshidrotic eczema or pompholyx that have led the author to suggest the condition is an atopic manifestation. It is similar in form to many of the continental MD or PhD Thesis supplements to dermatological journals.

Chapter 1 is a historical review of the numerous theories for the pathogenesis of pompholyx. The next chapter describes the clinical features of 58 patients with the disease including such aspects as sex, age incidence, smoking habits, trigger factors and its psychological consequences. Chapters 3 and 4 contain some studies on the pathogenesis. The former covers numerous topics ranging from thermography to chemical mediators, whereas the latter details atopic manifestations such as IgE levels and eosinophil levels. Within these chapters each topic is organized as a short paper with introduction, methods, results and discussion. Chapter 5 serves as a general discussion of the experimental and chemical studies. Subsequently, chapter 6 comprises two short papers on the use of topical PUVA and oral etretinate as therapy for the condition. Finally, a short discussion outlines some possible directions for future research.

This compilation includes a large amount of experimental detail but really has few conclusions for the non-researcher. It is a difficult book to read and is probably best approached as one would a group of papers, reading each individually rather than as a complete review of the subject.

It is most likely a book that should be available in dermatology departments and may be of interest to researchers who want a recent update on studies undertaken in this disease before they undertake their own thesis on the subject. However, I feel the dermatological clinician who is not interested in experimental details would not suffer from having missed it.

C.Holden

Pathology and Pathophysiology of AIDS and HIV-related diseases. Edited by S.J.Harawi and C.J.O'Hara (1989). London: Chapman and Hall Ltd. Pp. 512. Price £75.00.

This excellent book comprehensively reviews the epidemiology, immunology and virology of AIDS and HIV infection in its opening chapters, and in subsequent chapters deals with the manifestations of HIV infection in specific organ systems. In a rapidly progressing field the text is as up to date as possible with references up to mid-1988. The introductory chapter clearly reviews the basic immunology of HIV infection and provides a solid foundation for the later systemic chapters, particularly the chapter on the lymphoid and haemopoietic system. There are also excellent chapters on HIV infection in children, in Africa and related viral infections in non-human primates.

This book should appeal primarily to pathologists and it is therefore a pity that in contrast to the uniformly high quality of the text, that of the photomicrographs is variable in some chapters. With this one reservation, however, I can highly recommend this book to pathologists and indeed to anyone interested in the basic mechanisms underlying the widely varied clinical manifestations of HIV-related diseases.

M.Seywright

Cutaneous Investigation in Health and Diseases: Non-invasive Methods and Instrumentation. Edited by JEAN-LUC LEVEQUE (1989). New York: Marcel Dekker, Inc. Pp. 439. ISBN 0-8247-7967-3. Price £150.00.

Jean-Luc Leveque is well known for his work in the field of cutaneous bio-engineering and biophysics. In this book he has assembled a collection of articles to review the state of the art with respect to non-invasive techniques for cutaneous measurement. Most authors are from continental Europe, some are scientists, some are clinicians but all are experienced experts within the field of cutaneous measurement. Despite its title this book is not going to help the clinician with a difficult diagnosis. It is, however, a useful collection of many helpful critical reviews which direct the investigator towards the 'best buy'. There is some duplication but this helps by increasing the spectrum of advice rather than confusing. There are 16 chapters which contain just over a thousand references and although there is some lack of uniformity in referencing styles this is a valuable source of information for the researcher starting in these fields. I would recommend this book for the departmental library.

N.B.SIMPSON

The Biology of Wool and Hair. Edited by G.E.ROGERS, P.J.REIS, K.A.WARD AND R.C.MARSHALL (1989). London and New York: Chapman and Hall. Pp.506. ISBN 0-412-3 2120-3. £40·00.

The contents of this volume arose from a symposium held in Adelaide in 1987 which I attended, and the editors are to be congratulated on publication within 18 months. It follows a similar conference in Australia in 1964 whose proceedings were edited by Lyne and Short. Although the aim was to provide a survey of current knowledge in relation to wool biology, the contents provide a timely 'update' for all researchers on skin and hair. The topics covered include not only cellular and molecular processes in the follicle and epidermis, but also physiology and nutrition. A particular feature is the formation and role of the cytoskeleton in the growth of epidermal and follicular cells.

The first section: 'Structure and Function of the Hair Follicle' contains a paper on morphogenesis of the human hair follicle, which has no reference to similar work in sheep of 30 to 50 years ago. Another paper describes the way in which micro-surgery is enabling separation of the papilla and the study of its cells and the substances it produces—not only will transplanted papillae induce follicle development from the epidermis, but papilla cells grown in tissue culture will do so. The next group of six papers on 'Keratin Proteins' includes their variants as well as the latest in amino acid sequencing. The section on 'Physiological Factors' includes a convincing paper on the stimulation of human hair growth by the drug minoxidil, which has since been released and gone the way of previous 'cures' for baldness. Indeed a poster at the conference showed that minoxidil depressed wool growth. The fourth section covers 'Aberrations' and five papers on 'Growth and Differentiation' include one which showed that epidermal growth factor while stimulating the epidermis and sebaceous glands, depressed wool growth.

The real breakthrough since 1964, however, comes in the section on 'Keratin Genes', and as a wool researcher I find it gratifying that the first genes isolated come from wool not only in the context of skin, but in the context of agriculture. The transfer of the mouse growth hormone gene into sheep embryos with a subsequent increased rate of wool growth provides one glimpse into 'The Future', the title of the last group of eight papers. There is a good index.

M.L.RYDER

Models in Dermatology. Edited by H.I.MAIBACH AND N.J.LANE (1989). Vol. 4. Basel: Karger. Pp. 297. ISBN 3-8055-4761-7. Price £145.00.

This multi-author book is the fourth in a series of wide-ranging *in vitro*, animal and human model systems in dermatology. The areas covered include cell biology, dermatopharmacology, dermatotoxicology, metabolism, percutaneous penetration, physiology, immunology, disease models and wound healing, which are similar to those of previous volumes.

The articles are essentially a mix of clinical and basic science investigations using various model systems rather than reports elaborating on the pros and cons of the techniques involved in the use of different model systems, as the title may suggest.

The book will appeal to academic departments rather than to individuals, however I feel that it would have been more useful had each volume in the series been more specialized, such as one volume concentrating on the use of *in vitro* skin models and another on the use of animal systems. As it stands, the majority of the articles are too wide-ranging and short, although they do provide an excellent source of references.

The subjects covered in this book are short and very diverse. Relevant information required may be as easily obtained by scanning the literature as the price of this book seems rather excessive.

M.EDWARD

Oral Pathology. I.VAN DER WAAL AND W.A.M.VAN DER KWAST (1988). Berlin: Quintessence Publishing Company, Inc. Pp. 392, 355 illustrations. ISBN 0-86715-197-8. Price £70.50.

This is a beautifully produced book, very well illustrated with a large number of excellent clinical pictures and a smaller number of radiographs and photomicrographs. The clinical views and the photomicrographs are all in colour and with very few exceptions these are of high quality.

The intended readership is dentists, physicians and students and it is hoped that it will be helpful to others involved in the diagnosis of oral lesions. The coverage is certainly wide enough to be of interest to that readership and there are no significant omissions in content. The coverage of many areas is rather superficial, however, and the authors frequently state that they cannot go into more detail.

A large number of references (2377) are cited although many of these are not recent. The index has largely been compiled from section headings so that, while the main coverage of a topic is indexed, mention of a lesion in the differential diagnosis of another condition is often not indexed.

The book is a translation of the German version by the same authors published a year previously. The translation has not been well done. The standard of English in many areas is poor. Some of the terminology is not English and the text in areas is so badly rendered that it is not possible to determine what the authors are trying to express.

Overall, the impression is of disappointment that this most attractive appearing book does not have an equivalent quality of text. At its price it cannot really be strongly recommended for any of the intended categories of reader.

GORDON MACDONALD

Food Allergy. A Practical Approach to Diagnosis and Management. (Volume 5 in the series '**Clinical Pediatrics**'). Edited by L.T.CHIARAMONTE, A.T.SCHNEIDER AND F.LIFSHITZ (1988). Basel: Marcel Dekker Inc. Pp. 482. ISBN 0-8247-7652-6. Price $119.50.

British dermatologists are notably sceptical about food allergy, especially in relation to atopic dermatitis. Alas, this book can only serve to reinforce their prejudice. Allegedly a book about practical management, the book opens with the usual obligatory attempts (four chapters) to explain the 'basic mechanisms', and this is followed by four further chapters discussing causative agents. The five chapters on the clinical picture include one on 'skin manifestations', an especially poor section which exemplifies the central weaknesses of this book. In short, it is the uncritical regurgitation of the literature, and the failure to sift through the evidence, which so undermine the value of this and most other chapters. The reader is told, for example, that various foods and other items cause, aggravate, exacerbate or are implicated (the terms appear to be interchangeable) in chronic urticaria. The authors' task should have been to warn that most of the studies in this field (especially where food additives are concerned) are of little value, being based on a design where withdrawal of antihistamine immediately preceded challenges first with placebo and second with the substance under investigation. The mere listing of all possible food triggers is unhelpful, and this is compounded by the omission of reference to the one really key study by Supramanian and Warner (*Lancet* 1986; **ii**: 907–9). Whilst I would be happy to defend the use of elimination diets in a small proportion of children with atopic eczema, the notion that simple milk and egg free diets 'dramatically improve' 66–80% of patients with atopic dermatitis is ludicrous, and based entirely on the selective quotation of favourable studies. The book concludes with seven chapters on diagnostic tests, the prevention of food allergy and management. There is an appendix comprising a further seven chapters on diets for the avoidance of selected food items, so-called hypoallergenic formulae and food challenges. The chapter on 'immunologic tests' includes a particularly inadequate half page assessment of the value of skin tests. There is no question that this North American multi-author book represents a serious attempt to portray a balanced view of a difficult topic, and in this it is better than much that has come before. Readers of this contentious subject, from whatever background, however, desperately need to be able to distinguish between hard fact, anecdotal observation and speculation, and this is only achieved in a small number of chapters (notably the one on clinical methods of diagnosis).

T.J.DAVID

Diagnosis and Treatment of Common Skin Diseases. K.BORK AND W.BRAUNINGER (1988). Translated from the original German text by K.H.MUELLER AND H.K.MUELLER. Philadelphia: W.B.Saunders. Pp. 240, 428 colour clinical photographs. ISBN 0-7216-2240-2. £60.00.

This is a translation, the original book was primarily intended for the German equivalent of our general practitioner. It is in five sections: the common skin diseases (the largest section), sexually transmitted diseases, benign tumours, malignant tumours and treatment. Each of the first four sections is subdivided into topics such as acne, erysipelas, squamous cell tumours and so on. Most subjects are covered in two pages with four illustrations on the left hand page and the supporting text on the right. The authors' aim is to give a synopsis of the clinical aspects and treatment with special emphasis on the illustrations. Illustrations are important in dermatology and these are particularly good. The book is well presented with concise text. However, though the outlines of treatment are covered, insufficient details are given and no attempt has been made, in section 5, to substitute British for German preparations (though the American equivalent where available has been given).

The book is primarily an atlas of dermatology and as such could be very useful in diagnosis. However, I doubt whether the best form of presentation is used. In the original German edition the subjects were dealt with in alphabetical order. However, the order is unchanged for the English version so the diseases no longer remain in strict alphabetical order. In addition, I am not convinced this approach is the best one for an atlas as it presupposes that the reader has already made the correct diagnosis. The problem is compounded here as no attempt has been made to include a differential diagnosis.

In summary, the book has excellent illustrations, a major asset but the format of the book and therapeutics section could be greatly improved.

R.LEVER

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News and Notices

Metabolic Disorders and Nutrition related to the Skin, Utrecht, 8–10 March 1990

A clinically orientated symposium on Metabolic Disorders and Nutrition related to the Skin, organized jointly by the European Society for Dermatological Research and the Netherlands Society of Dermatology and Venereology, will be held in Utrecht, the Netherlands, from 8 to 10 March 1990. For further information contact: Dr Willem A. van Vloten, Department of Dermatology, University Hospital Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands.

Institute of Dermatology, University of London

Professor Malcolm W. Greaves has been appointed as Dean of the Institute of Dermatology with effect from 1 October 1989.

British Journal of Dermatology (1989) 121, 551–562.

A study of persistent photosensitivity as a sequel of the prior administration of the drug benoxaprofen

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SUMMARY

Cutaneous photosensitivity is a recognized side-effect of a number of commonly used groups of drugs, among which are the non-steroidal anti-inflammatory agents (NSAIDs) one of which, benoxaprofen, was withdrawn from use in 1982. The abnormal reaction of the skin to light usually occurs during the period of the systemic administration of the drug, but may with some drugs persist for longer, even for months. Chronic photosensitivity, i.e. 'persistent light reaction' of some years duration, continuing after the withdrawal of the primary cause, has so far only been reliably reported when the initial exposure of the skin to the photoactive substance has been by external contact and not following ingestion. A suggestion that photosensitivity could persist for a period of years as a sequel of initial benoxaprofen-induced photosensitivity was studied in a group of 42 subjects said to be affected in this way. The results failed to confirm the presence of persistent photosensitivity. It appeared that the probable explanation for the episodic abnormal reactions to light over the years since 1982 was the systemic administration of other photoactive drugs including a series of NSAIDs.

The ability of an increasing number of commonly used drugs such as the phenothiazines, the sulphonamides, the tetracyclines, and the non-steroidal anti-inflammatory agents (NSAIDs), to cause cutaneous photosensitivity as an unwanted side-effect has led to the study of the clinical features and the course of such reactions, and to the identification by phototesting with the responsible wavelengths of light. Abnormal reactions of the exposed skin are caused by a variety of environmental factors, only one of which is 'light', and it is therefore often necessary to confirm the diagnosis by phototesting. Identification of the responsible wavelengths by phototesting is useful not only in indicating the specific photodermatosis, but also, in the case of it being drug induced, which drugs are likely to be responsible. The diagnosis of drug-induced photosensitivity is based on the recognition of the clinical features which suggest the presence of photosensitivity,[1] followed by the selection of the specific form of photosensitivity present on the basis of morphological and other features. In this way drug-induced photosensitivity rather than one of the other photodermatoses can be identified with reasonable certainty.

Phototesting techniques have shown that with some drugs and in some individuals the photosensitivity may continue for a variable period after the drug has been withdrawn, but only for a period of months and not years.[2-5] It had been suggested, however, that one of the NSAIDs, benoxaprofen, was different in that in some instances photosensitivity could persist for years after the drug had been withdrawn.[6] Therefore, during 1987 we studied a group of 42 patients who gave a history of continuing photosensitivity since the drug was withdrawn in 1982. Most of these patients had originally suffered from benoxaprofen-induced photosensitivity. An earlier study had defined the action spectrum of the photosensitivity due to this drug.[7] The publication of the results of this 1987 study have been delayed since they formed part of a legal report which until recently was *sub-judice*.

METHODS

Subjects

The 42 subjects studied (Table 1) were unselected except in so far as they were involved in litigation and had given a history of continuing photosensitivity over the years following benoxaprofen administration. For most patients, their medical records dating back to 1980 were obtained although they were not always complete. The details of cutaneous photosensitivity and drug therapy were noted. This information was supplemented by direct discussion prior to phototesting at the time of the examination of the skin for evidence of cutaneous photosensitivity.

Phototesting

The techniques used have been described elsewhere[8-10] and made use of both monochromator and solar simulator irradiation sources. They consisted of exposure of the back skin to a range of measured doses from a series of wavebands throughout the solar spectrum from 290 to 700 nm. Recordings were made of symptoms (irritation, pricking, burning, pain, etc.) and signs (erythema, urtication, etc.) at the following time intervals after irradiation: 0, 5, 10, 15, 30 min and at 7, 24 and 48 h. Any reactions were compared to those known to occur on the back skin of normal non-photosensitive subjects similarly exposed and in this way abnormality was detected, both for immediate and delayed reactions, and the responsible wavelengths identified. The programme (Table 2) was planned to follow closely that of the original group of subjects studied in 1981 when under treatment with benoxaprofen and which had led to the definition of the action spectrum of the induced photosensitivity.[7] It also covered the programmes routinely used in the unit for the diagnosis of suspect photosensitivity of all kinds,[10] including drug-induced.[4] It differed by the addition of a number of extra wavebands and a series of higher doses which had been shown to be relevant in the 1981 study (indicated by* in Table 2). However, as the study progressed it was found that some of these higher doses tended to produce unwanted delayed persistent erythema reactions and therefore they were not used for all of the group and are marked as such by ** (Table 2).

RESULTS

Of the 42 subjects studied during 1987 there was reference in their medical records (Table 1) in 14 of them, although sometimes only a single observation, to photosensitivity occurring during the time when they were taking benoxaprofen. However, in only four (nos 10, 16, 31 and 35) was there reference to photosensitivity for the period from 1982 to 1987 after the benoxaprofen had been withdrawn, and in fact, of these four cases, no. 16 had been taking a potentially photoactive drug, a thiazide diuretic hydrochlorthiazide, regularly for many years; no. 10 had also been

TABLE 1. Subject histories

			Reference in medical records to photosensitivity		History of photosensitivity at 1987 assessment		
Case no.	Sex	Duration of benoxaprofen therapy	During benoxaprofen therapy	Post-benoxaprofen therapy	During benoxaprofen therapy	Post-benoxaprofen therapy	Results of phototesting
1	F	April 81–April 82	+	–	+	Atypical	N
2	F	May-August 1982	–	–	+	+	N
3	F	April 81–July 82	+	–	+	+	N
4	M	June 81–Aug. 82	–	–	–	+	N
5	F	June–December 81	–	–	+	Atypical	N
6	F	June 81–June 82	–	–	+	Atypical	N
7	F	June 81–Feb. 83	–	–	+	–	Abnormal: 335 nm FN
8	F	Nov. 80–June 81	+	–	+	+	N
9	M	May 82	–	–	–	Atypical	N
10	F	May 82	+	+	+	Atypical	N
11	F	? June–Aug. 81	?	?	–	+	Abnormal: 305, 310, 335, 400 nm
12	F	Jan. 81–May 83	–	–	+	+	N
13	M	? 1981/82	Not available		+	Atypical	N
14	M	May 81–Aug. 82	+	–	+	+	N
15	F	June–July 82	–	–	+	Atypical	N
16	F	Nov. 80–Sept. 82	–	+	+	+	Abnormal: 335 nm H
17	M	March 81–April 82	+	–	+	–	N
18	F	1981/2	?+	–	?+	–	N
19	F	1981 (?April–Aug.)	Incomplete records		?+	?+	Abnormal: 335, 400 nm
20	F	? Oct. 81–Aug. 82	Incomplete records		+	+	Abnormal sensations; not reproducible
21	F	May–June 82	?+	–	?+	–	Not tested
22	M	April–Aug. 82	–	–	+	+	N
23	F	Aug.–Oct. 81	–	–	?+	Atypical	N
24	F	? 1981/2	–	–	+	+	Abnormal sensations; not reproducible
25	M	Sept. 81–May 82	–	–	–	–	N
26	F	May 80–Aug. 82	–	–	unclear history		N
27	F	April 81–May 82	+	–	+	+	N
28	F	Oct. 81–June 82	–	–	+	+	N
29	F	May 81–April 82	–	–	+	+	Abnormal: 310, 315, 320, 325, 330 nm TI H
30	M	Nov. 80–Aug. 81	–	–	+	Atypical	N
31	F	Sept. 81–Aug. 82	?+	?+	+	Atypical	N
32	F	Dec. 80–Aug. 82	–	–	+	?+	N
33	F	March 81–Aug. 82	–	–	+	?+	Abnormal: 400 nm M
34	F	1981/82	–	–	–	–	N
35	M	Nov. 81–Aug. 82	+	+	+	+	Abnormal: UVB/UVA PD
36	M	April–May 82	+	–	+	–	N
37	F	Jan.–March 82	–	–	+	+	N
38	F	Feb.–July 82	–	–	? no/yes atypical		N
39	M	Feb.–Aug. 82	No records available		+	+	N
40	F	Jan. 81–March 82	+	–	+ atypical	–	N
41	M	Dec. 80–April 82	?+	–	+	+	N
42	F	1980–?	–	–	+	?+	Abnormal: 400 nm FR IB

Wavebands
305 ± 5 nm.
310 ± 5 nm.
315 ± 5 nm.
320 ± 5 nm.
325 ± 5 nm.
330 ± 5 nm.
335 ± 30 nm.
400 ± 30 nm.

Drug therapy
FN, Fenbrufen.
FR, Frusemide.
H, Hydrochlorthiazide.
IB, Ibuprofen.
M, Mefenamic acid.
TI, Tiaprofenic acid.

+, Positive information.
–, Negative information.
N, Normal.
PD, Photosensitivity dermatitis.

TABLE 2. Phototesting programme

Irradiation source	Waveband	Dose
Monochromator	305 (±5 nm)	8·2 (mJ/cm²)
		22
		56*
		150* **
		390* **
		820* **
	310 (±5 nm)	39* (mJ/cm²)
		100*
		220*
		470* **
		820* **
	315 (±5 nm)	100* (mJ/cm²)
		220*
		470*
		1200* **
	320 (±5 nm)	100* (mJ/cm²)
		220*
		470*
		1200*
		2200* **
	325 (±5 nm)	100* (mJ/cm²)
		220*
		470*
		1200*
		2200*
	330 (±5 nm)	100* (mJ/cm²)
		220*
		470*
		1200*
		2200*
	340 (±5 nm)	470* (mJ/cm²)
		1200*
		2200*

Irradiation source	Waveband	Dose
	360 (±5 nm)	470* (mJ/cm²)
		1200*
		2200*
	335 (±30 nm)	470 (mJ/cm²)
		1200
		3300
		6800*
	365 (±30 nm)	1200 (mJ/cm²)
		2700
		5600
		12 000
	400 (±30 nm)	4700 (mJ/cm²)
		10 000
		22 000
		47 000
	430 (±30 nm)	10 000 (mJ/cm²)
		22 000
		47 000
		82 000
Solar simulator	290–700 nm	5 (J/cm²)
		10
		15
		20*
		25* **
		30* **
	320–700 nm	20 (J/cm²)
		40
		70
		100
		170
		200*

The minimal response dose (MRD) for immediate and delayed erythema and for urtication or abnormal sensations was recorded on day 1, and on day 2 the interval between the minimal response dose and the neighbouring no response dose was filled in by a further range of doses so as to determine the MRD as accurately as possible. Also on day 2 if no abnormal immediate responses had been noted with the highest doses at wavebands 315, 320 and 325 nm, the skin was then irradiated with the following single doses at these wavebands: 315 nm (2500 mJ/cm²), 320 nm (2850 mJ/cm²), 325 nm (2500 mJ/cm²).

The wavebands and dosages marked with an asterisk were additional to those used in the routine diagnostic phototesting programme and were included for comparison with those which had been shown to produce abnormal reactions in the the 1981 study.[7] A few of the highest dosages, those marked**, were not used in all cases as explained in the text.

Filters:

WG305 used with wavebands 360, 335 and 365 nm.

WG345 used with wavebands 400, 430 nm.

Copper sulphate and WG305 used with solar simulator wavebands.

290–700 nm and WG345 used with waveband 320–700 nm.

prescribed a variety of potentially photoactive drugs (Table 3); and no. 35 was subsequently found to be suffering from photosensitivity dermatitis which was not due to a systemically administered drug.

At the time of the 1987 assessment a retrospective history was obtained which was compatible with photosensitivity having occurred in 36 out of the 42 subjects during the benoxaprofen administration period in 1981–82 (Table 1). This was of variable severity and duration and was in fact doubtful in five of the 36. Also two were recognized as being affected by one or other of the chronic photodermatoses additional to any possible drug induced component. What was less clear during the 1987 assessment was the diagnosis of the possible photosensitivity in the years following the withdrawal of benoxaprofen, although in 25 subjects (Table 1) a variable history was obtained compatible with photosensitivity having occurred at different times since 1982. Of these, three were noted to have vitiligo (case nos 4, 24 and 29) and one suffered from a chronic photosensitivity dermatitis (case no. 35). Perhaps the most significant finding was that the majority were under treatment with one or other of a range of potentially photoactive drugs (Table 3) at various times from 1981 to 1987, covering both the benoxaprofen administration period of 1981–82 and subsequently up until the assessment in 1987. Some also received such drugs at the same time as the benoxaprofen, either immediately before starting or after withdrawal, and in a few instances (nos 3, 7, 31, 32 and 42) at the same time as they were taking benoxaprofen.

The results of phototesting (Table 4) were of interest in that in 31 out of 41 subjects (no. 21 was not tested), results were normal (Table 1). Of the remaining 10 in whom some abnormality was detected, in two (nos 20 and 24) it was confined to some discomfort of the skin which was not reproducible, and no. 35 suffered from a separate photodermatosis, photosensitivity dermatitis. Of the remaining seven subjects, abnormal immediate or delayed erythema was recorded at a single (nos 7, 16, 33 and 42) or at multiple wavebands (nos 11, 19 and 29) (Table 1); however, all of these, with the exception of case nos. 11 and 19, were being treated at the time of testing with other potentially photoactive drugs such as fenbufen (no. 7), hydrochlorthiazide (no. 16), mefenamic acid (no. 33) and no. 42 was taking both frusemide and ibuprofen and case no. 29 both tiaprofenic acid and hydrochlorthiazide (Table 3). Thus, there was an explanation for the abnormal findings in five of these remaining seven subjects, the exceptions being case nos 11 and 19, further assessment of whom was not possible in 1987 for a variety of reasons.

DISCUSSION

This study failed to confirm the presence of photosensitivity in the majority of the subjects despite a history of reacting to light since the withdrawal of benoxaprofen in 1982. The abnormal reactions of the skin to light which the patients described were probably episodic and related to the administration of other known photoactive drugs, and in particular other NSAIDs.

There is no evidence that photosensitivity, based on a phototoxic mechanism, can persist for years after the withdrawal of the drug. This persistence has been reported when the skin has been exposed to a photoactive substance by external contact, with the subsequent development of a state of 'persistent light reaction' such as that seen in the photosensitivity dermatitis and in the actinic reticuloid syndrome. However, this is thought to involve immunological mechanisms, whereas there is little, if any, evidence for such mechanisms, i.e. 'photoallergy', in the photosensitivity reactions following systemic administration of the drugs (Table 5). Photoallergy has, however, been induced in mice to both sulphanilimide and chlorpromazine.[11]

TABLE 3. Potentially photoactive drug treatment other than with benoxaprofen, received during the years 1982–87

Case number	Drug*	Period when drug taken
1	Nil	
2	H	1977–87
	P	1982–83
	IB	1983
	FR	1983
	IN	1983
3	NA	1981, 1985–87
	AZ	1982
	FL	1981
	P	1982
	K	1983, 84, 85–87
4	D	1984–85
5	P	1982
	FL	1984
	Q	1985–86
6	NA	1981, 82–87
	FL	1981, 82, 83
	Q	1983
7	K	1982–85
	IB	1982, 83
	FN	1983, 84, 85–87
8	NA	1981
	DI	1980, 83
	P	1981, 83, 85
	AZ	1982
	SU	1982, 83, 84, 86
	P	1983
	FN	1984
	K	1986
9	B	1982, 83
10	CH	1982
	NA	1986–87
	P	1985, 86
	H	1985, 86
11	K	1981
	B	?
12	P	1983, 84, 85
	NA	1986
	FN	1985
13	No records	
14	A	1982, 83, 84, 85
	P	1982, 83, 85
15	Nil	
16	H	1982–87
17	No records post-1983	
18	P	1983, 84
	NA	1983, 85
	AZ	1985
	FL	1986
19	? incomplete records	
20	IB	1983, 84
	NA	1983
	D	1985, 86–87
21	P	1982
	H	1983, 84
22	P	1982
	FL	1982
	NA	1982
23	P	1982–87
	FN	1982
24	NA	1982–87
	H	1984
	IB	1984
	D	1982, 84
	NAL	1981
	FN	1982, 83
25	FE	1981
	IB	1982
	FN	1982
26	FR	1981, 84
	B	1985
	H	1982
	P	1981
	D	1982
27	NA	1984–86
	SU	1983, 84
	AZ	1984
28	P	1984–87
	NA	1982, 83
	FN	1983
	K	1983
29	FN	1982, 84
	P	1982
	K	1982
	FE	1983
	P	1984, 85
	H	1983, 87
	IB	1985
	TI	1984

TABLE 3 (*contd*)

Case number	Drug*	Period when drug taken
30	Incomplete records; P, IB, FN, AZ, K, FL at various times	
31	IB	1981, 83
	NA	?1982
	K	?1982
32	P	1982
	D	1982, 83, 84 85, 86–87
	NA	1985
33	Incomplete records	
	P	1982, 83
34	P	1981, 82, 84
	FL	1985
	FN	1985
	A	1985, ?87
	B	1981
35	FN	1982–84
36	NA	1982
37	IB	1982
	N	1982, 83, ?85
38	N	1982–87
	Q	1983
	FL	1981, 82, 83
	B	1981
	P	1982–87–?
	TE	1982, 83, 84
	H	1984
	Q	1985, 86
39	P	1982, 83
	NA	1983
	A	1983
	D	1983, 84, 85
40	IB	1982, 83, 84, 86, 87
41	Nil	
42	H	1981, 82, 83, 84
	NA	1982
	A	1982
	IB	1983, 85, 86

* Azapropazone (A); Bendrofluazide (B); Chlorpromazine (CH); Diclofenac (D); Fenoprufen (FE); Fenbufen (FN); Fluribiprofen (FL); Frusemide (FR); Hydrochlorthiazide (H); Ibuprofen (IB); Indoprofen (IN); Ketoprofen (K); Mefenamic acid (M); Nalidixic acid (NAL); Naproxen (NA); Piroxicam (P); Quinine Sulphate (Q); Sulphasalazine (SU); Tetracycline (TE); Tiaprofenic Acid (TI).

Benoxaprofen was widely used during 1980–82 for the treatment of arthritis but was withdrawn due to unwanted side-effects, the most common being cutaneous photosensitivity. The reported incidence of this photosensitivity varied,[7,12] probably due to factors such as skin type, amount of sunlight exposure, the dosage of the drug taken, and the way the individual subject dealt with the dosage regime, leading to variable amounts of the photoactive substance reaching the skin. The mechanism involved was thought to be a phototoxic one directed against the cell membrane, and probably mediated by a photoproduct rather than the parent substance.[13] The clinical features consisted mainly of symptoms (burning, pricking, and painful discomfort) rather than signs (erythema, urtication, etc.), the discomfort arising early on so that those affected tended to come out of the sunlight before any change in the skin, other than minimal erythema, was noticed. More severe reactions occurred where further exposure to sunlight was unavoidable and if appropriate topical photoprotection was not used.[14] The responsible wavelengths (the action spectrum) for the photosensitivity reaction were shown to be within the ultraviolet waveband from 310 to 325 nm with a peak activity at 315–320 nm.[7] These episodic reactions of the skin usually tended to clear within a few hours after coming out of the sunshine.

TABLE 4. Minimal-erythema-dose estimations

	Case number	Waveband 305 (±5 nm)	310 (±5 nm)	315 (±5 nm)	320 (±5 nm)	325 (±5 nm)	330 (±5 nm)	340 (±5 nm)	360 (±5 nm)	335 (±30 nm)	365 (±30 nm)	400 (±30 nm)	430 (±30 nm)	290–700 nm	320–700 nm
Immediate erythema															
	7	>47	NT	NT	>2200	NT	NT	NT	NT	3300*	>12000	>47000	>82000	>10 J	>200 J
	11	390*	820*	2500	>2850	>2500	>2200	>2200	>2200	6800*	12000	27000*	>82000	>30 J	>170 J
	16	>47	NT	NT	NT	NT	NT	NT	NT	4700*	>12000	>47000	>82000	NT	NT
	19	820	>820	2500	>2850	>2500	>2200	>2200	>2200	4700*	>12000	>47000	>82000	30 J	100 J
	33	>56	>220	>470	>1200	>2200	>2200	>2200	>2200	>6800	>12000	27000*	>82000	>20 J	>200 J
	42	>56	>220	>470	>1200	>2200	>2200	>2200	>2200	>6800	>12000	33000*	>82000	>20 J	170 J
All others		>820	>820	>2500	>2850	>2500	>2200	>2200	>2200	>6800	>12000	>47000	>82000	>30 J	>200 J
Normal values (lowest normal)		820	>820	>1200	2200	>2200	>2200	>2200	>4700	>6800	12000	47000	82000	30 J	47 J
Delayed erythema															
	19	56	180	1200	2850	>2500	>2200	>2200	>2200	4700	8200*	33000	>82000	10 J	200 J
	29	>56	220	220*	680*	470*	680*	>2200	>2200	6800	>12000	>47000	>82000	>20 J	>200 J
	35	2·7*	39*	100*	220*	220*	470*	2200	>2200	100*	2700*	>47000	>82000	1 J*	>200 J
All others		27– >82	150– >330	330– >680	1000– >1200	1200– >2200	1200– >2200	1500– >2200	>2200	1800– >6800	8200– >22000	>47000	>82000	7– >15 J/cm^2	70– 200 J/cm^2
Normal values Range (mJ/cm^2)		47–82	180– 330	390– >680	>1200	>2200	>2200	>2200	>2200	3300– 6800	12000– 22000	47000– >47000	>82000	8·2– 15 J/cm^2	100– 200 J/cm^2
Lowest dose (mJ/cm^2)		27	150	330	1000	1200	1200	1500	>2200	1800	8200	47000	>8200	7 J/cm^2	70 J/cm^2

* Indicates abnormal reading.

NT = Not tested.

Immediate erythema is defined as erythema lasting longer than 5 min.

The range of normal values is that within which 70% of the normal subjects tested responded.

Doses are in mJ/cm^2 except for wavebands 290–700 and 320–700 nm where they are in J/cm^2.

TABLE 5. Drugs which may cause cutaneous photosensitivity

- Amiodarone
- Frusemide
- Nalidixic Acid
- NSAIDs
  - Azapropazone
  - Diclofenac
  - Fluribrufen
  - Ibuprofen
  - Ketoprofen
  - Naproxen
  - Piroxicam
  - Tiaprofenic Acid
- Phenothiazines
- Quinine
- Retinoids
- Sulphonamides
  - Thiazide Diuretics
- Tetracyclines

TABLE 6.

Case no.	Sex	Age (years)	Monochromator irradiation at waveband 320 ± 5 nm	
			Minimal dose for immediate erythema	
			In 1982	In 1985
1	F	?	330 mJ/cm^2*	NT
2	F	65	1000*	> 1000 mJ/cm^2
3	F	49	560*	> 1000
4	F	59	1000*	> 1000
5	F	64	2200	> 1000
6	F	?	820*	NT
7	M	64	560*	> 1000
8	F	65	680*	> 1000
9	M	69	1500	> 1000
10	M	57	2200	> 1000

Immediate erythema is defined as erythema lasting for longer than 5 min.
NT, Not tested.
*, Abnormal levels.
Normal values for immediate erythema at 320 ± 5 nm is > 1000 mJ/cm^2.

TABLE 7. Phototoxic potential of NSAIDs using a battery of *in vivo* tests

Positive	Positive at high concentrations	Negative
Tiaprofenic acid	Azapropazone	Piroxicam
Benoxaprofen	Diclofenac	Sulindac
Ketoprofen	Fenoprofen	Indomethacin
Fenbrufen	Flurbiprofen	
Carprofen	Ibuprofen	
Naproxen		
Suprofen		

It has been suggested since the epidemic of the early 1960s of contact and photocontact dermatitis to the halogenated salicylanilides[15] that some cases of photosensitivity develop a state of a persistent light reaction. It was proposed that the original salicylanilide-induced photosensitivity reaction changed the skin in such a way that it continued to have the potential for reacting abnormally to light without further exposure to the primary photosensitizer.[16,17] The alternative explanation is that the persisting photosensitivity was due to continuing undetected exposure to the original photactive substance and/or allied or other photoactive substances, and perhaps it was more common in skin affected by long-term chronic inflammatory responses, due at least in part to a cell-mediated contact allergy.[18] The substances incriminated in this production of persistent photosensitivity are known to be both contact allergens as well as photosensitizers.[18-22]

A significant finding of this study, contrary to the diagnosis of persistent photosensitivity induced by benoxaprofen, is the normal phototests in the majority of cases (31 out of 41–76%) as carried out in 1987. Also, the abnormalities did not show a broad spectrum of response [23-25] but were usually restricted to a single waveband and were often associated with the current administration of a photoactive drug. In a group of subjects who had previously been phototested in 1982 whilst taking benoxaprofen, the reaction at the 320 nm waveband, which was the peak of the abnormal response[7] in these subjects, had returned to normal by 1985 (Table 6) (H.A.Addo, J.Hosie, W.Frain-Bell 1985, unpublished communication). The two reports which have suggested persistent photosensitivity of some years' duration, have been incomplete in that either phototesting was omitted[26] or that alternative forms of cutaneous photosensitivity such as photosensitivity dermatitis had not been satisfactorily ruled out.[26,27]

The history taken at the time of the 1987 assessment indicated that a number had suffered continued photosensitivity. It was uncertain why this had happened in this group of patients and not others who had similarly experienced drug induced photosensitivity at the time of benoxaprofen administration. The episodic nature of the reactions which they described suggested that, given the absence of photosensitivity demonstrated by phototesting in 1987, these episodes were due to exposure at the time to a photoactive substance and probably an ingested one; and as can be seen in Table 3 all, with the exception of case nos. 15 and 41, had been taking one or more of a range of photoactive drugs, particularly other NSAIDs, over the years since 1982. There are reports of clinical photosensitivity resulting from the administration of piroxicam,[26,28-33] naproxen,[34-38] azapropazone,[39] and two studies which reported the induction of photosensitivity as demonstrated by abnormal phototest results after the administration of both piroxicam and azopropazone.[40,41] Some of the NSAIDs in Table 5 have been reported with variable frequency to the Committee on Safety of Medicines as being associated with the development of cutaneous photosensitivity. In addition, laboratory investigations using model systems such as photohaemolysis,[42,43] the release of histamine from basophils,[44] the photosensitized killing of *Candida albicans*[33,43] and mouse peritoneal macrophages,[42] inhibition of PHA stimulated DNA synthesis in human lymphocytes,[42] and destruction of the amino acid histidine[42] demonstrated the phototoxic potential of a number of NSAIDs. In the unit in Dundee the results of screening 15 NSAIDs using a battery of tests[42] showed that the majority have a phototoxic potential even though high concentrations of the drug may be required in some instances (Table 7, B.E.Johnson 1988, personal communication). Using similar tests, the metabolites of both piroxicam[28] and sulindac (B.E.Johnson 1988, personal communication) have been shown to be phototoxic, adding to the complexity of interpretation when these different drugs have been used.

It would appear therefore that photosensitivity induced by ingested drugs may persist for some months after the drug has been discontinued but not for a period of years. Where patients have suffered from photosensitivity induced by a particular drug, such as benoxaprofen, and continue to have abnormal reactions to light in subsequent years, alternative reasons, especially the taking of other photoactive drugs should be considered.

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Identification of CD16/NKH-1$^+$ natural killer cells and their relevance to cutaneous tumour immunity

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SUMMARY

The presence of natural killer (NK) cells, as defined by reactivity with the monoclonal antibodies Leu 7, Leu 11 and Leu 19 was assessed in the inflammatory infiltrate around epidermal neoplasms and compared with findings in a range of inflammatory dermatoses. HNK-1$^+$ (Leu 7) cells were present in a wide range of malignant, pre-malignant and inflammatory disorders. Cells positive for the more specific NK cell antigens CD16 (Leu 11)/NKH-1 (Leu 19) had a distribution mainly restricted to cases of squamous cell carcinoma (five of nine) and keratoacanthoma (three of seven). The variability in distribution between the different antibodies suggests that the majority of cutaneous HNK-1$^+$ cells are not NK cells, but represent cross-reacting T lymphocytes. The qualitatively distinct distribution of CD16$^+$ and NKH-1$^+$ cells around some cases of squamous cell carcinoma and keratoacanthoma is of interest but their absence from a number of such cases calls into question a specific effector role for natural killer cells in these squamoproliferative tumours.

The frequent finding of a mononuclear cell infiltrate around cutaneous tumours[1,2] is suggestive of an immunological response which may play a role in rejection, control of metastases or prevention of local spread of tumour. The infiltrate is predominantly composed of T lymphocytes and macrophages, suggesting a central role for T-cell-mediated hypersensitivity mechanisms in tumour immunity. However, cytotoxic reactions involving natural killer (NK) cells, which do not require the presence of class I or class II major histocompatibility complex (MHC) antigens on target cells, may play an important role in tumour surveillance and rejection.[3–5]

NK cells are defined by their ability to lyse certain tumour cells *in vitro* in the absence of antibody or prior immunization.[6] They commonly express the cell surface antigens CD16 (Leu 11) and NKH-1 (Leu 19).[7] The CD16 antigen is present on cells with natural killer activity and some neutrophils.[8,9] The recently recognized antigen NKH-1[10,11] is present on the majority of

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resting and activated Leu 11$^+$ natural killer cells, together with a small population of CD 3$^+$ non-MHC restricted cytotoxic T lymphocytes (CTL). An additional antigen HNK-1 (Leu 7)[8,12] is found on 10–20% of peripheral blood lymphocytes (PBL), a proportion of which do show natural killer cell function. However, approximately 40% of Leu 7$^+$ cells co-express the T-cell antigens CD4 or CD8 which are not present on NK cells. In peripheral blood the majority of cells with natural killer activity have the phenotype CD3$^-$, CD16$^+$ (Leu 11), NKH-1$^+$ (Leu 19).[10]

The majority of immunohistochemical studies of cutaneous disorders to date have taken the HNK-1 antigen as indicating the presence of NK cells.[13–16] However, this antigen is not NK specific, and studies detecting CD16/NKH-1 antigens are required to clarify the role of NK cells in cutaneous disease. We utilized Leu 7, Leu 11 and Leu 19 monoclonal antibodies to analyse the infiltrate around a range of benign, pre-malignant and malignant epidermal tumours (squamous cell carcinoma, Bowen's disease, actinic keratosis, keratoacanthoma, basal cell carcinoma, basal cell papilloma, viral wart). We compared our results with those obtained in inflammatory dermatoses (lichen planus, psoriasis, discoid lupus erythematosus, atopic dermatitis, contact dermatitis) to establish the presence of NK cells and, if present, whether there are tumour specific patterns, which may support an *in vivo* role for NK cells in cutaneous tumour defence.

METHODS

Subjects

Skin biopsy was performed for diagnostic purposes following informed consent. An ellipse of skin was removed and bisected, half was routinely processed for histopathological assessment, the other half being stored in liquid nitrogen awaiting immunohistochemical analysis. Histopathological examination revealed: squamous cell carcinoma (nine cases), keratoacanthoma (seven cases), Bowen's disease (four cases), actinic keratosis (four cases), basal cell carcinoma (nine cases), basal cell papilloma (five cases), psoriasis (three cases), lichen planus (six cases), discoid lupus erythematosus (two cases), atopic dermatitis (four cases) and contact dermatitis (five cases).

Tissue processing

Tissue taken for immunohistochemical study was frozen in liquid nitrogen-cooled isopentane using OCT (Tissue Tec, Miles, TX, U.S.A.) cryoprotectant, and stored in liquid nitrogen at −70°C until used. Serial 5 μm cryostat sections were then mounted on glass slides; air-dried and fixed in acetone for 10 min at room temperature prior to staining.

Immunoperoxidase staining

Serial cryostat sections were subjected to a standard peroxidase–antiperoxidase method (PAP) as previously described.[17] Briefly, cryostat sections were incubated with 100 μl of optimal dilution of Leu 7, Leu 11 and Leu 19 respectively for 60 min and subsequently incubated with rabbit anti-mouse immunoglobulin for 30 min, followed by mouse peroxidase–antiperoxidase complexes (PAP) for 30 min (all non-primary antibodies supplied by Dako U.K., Ltd, High Wycombe, Bucks.). Primary antibodies used are shown in Table 1. Sections were washed between each step using Tris-buffered saline pH 7·6 and developed with diamino benzidine (DAB) reaction (10 mg of DAB in 10 ml Tris-HCl pH 7·6) for 10 min prior to counterstaining using Mayers haemalum and mounting in DPX (BDH, Poole, Dorset, U.K.). Cryostat sections

TABLE 1. Antibodies used and their characteristics

Antibody	Antigen	Dilution	Cell Reactivity
Leu 7	HNK-1 (110 kDa)	1:10	NK cells, CD4/CD8 T lymphocytes
Leu 11	CD16 (Fc receptor IgG)	1:10	NK cells, neutrophils
Leu 19	NKH-1 (220 kDa)	1:20	NK cells, $CD3^+$ T cell subset

* Antibody source—Becton Dickinson, Mountain view, California.

of human tonsil were used as positive controls. Negative controls comprised omission of appropriate primary and secondary antibodies, substitution of inappropriate monoclonal antibodies of similar isotypes, and staining with DAB alone.

Assessment

For each antibody, the percentage of positive staining cells per 200 mononuclear cells at × 500 magnification was assessed using an eyepiece graticule, and expressed semi-quantitatively as: < 10%, 10–50%, > 50%. Serial sections were examined using each antibody.

RESULTS

The presence of positive staining cells detected with each antibody in the various disease categories is summarized in Table 2. Only cells with definite membrane staining were included.

$HNK\text{-}1^+$ (Leu 7) cells were seen in examples of all tumours and inflammatory dermatoses. The majority of positive cells were randomly distributed throughout the tumour-associated infiltrate, although in basal cell carcinoma, small numbers of $HNK\text{-}1^+$ cells were seen within the tumour bulk. In no disorder did the positive cells constitute more than 10% of the infiltrate. Characteristic staining of cutaneous nerves with Leu 7[18,19] was frequently seen in the dermis and provided an internal positive control.

$CD16^+$ (Leu 11) cells were restricted to the infiltrate around squamous cell carcinoma (five of nine cases, Fig. 1) and keratoacanthoma (three of seven cases). The staining was less intense than with Leu 7 and positive cells never constituted more than 5% of the infiltrate. No $CD16^+$ cells were seen within tumour tissue. Variable staining of keratinocytes in the stratum granulosum by Leu 11 was noted, being maximal in the region of the acrosyringium (Fig. 2).

$NKH\text{-}1^+$ (Leu 19) cells, as with CD16, were mainly associated with squamous cell carcinoma (three of nine) and keratoacanthoma (two of seven) staining less than 5% of the infiltrate. Occasional positive cells were seen in single cases of Bowen's disease, basal cell carcinoma and contact dermatitis. Both double staining reactions and comparison of serial sections of lesions positive for both CD16 and NKH-1 suggested that some of the $NKH\text{-}1^+$ cells were $CD16^-$. Leu 19, like Leu 7, gave a strong staining reaction with cutaneous nerves (Fig. 3).

TABLE 2. Presence of NK cell antibody staining in the infiltrates around cutaneous tumours and in inflammatory dermatoses

	Total number of cases	Total number of positive cases	Presence of positive cells for each antibody (Number of cases)		
			Leu 7	Leu 11	Leu 19
Squamous cell carcinoma	9	6	+ (6)	+ (5)	+ (3)
Keratoacanthoma	7	6	+ (3)	+ (3)	+ (2)
Actinic keratosis	4	4	+ (4)	—	—
Bowen's disease	4	3	+ (3)	—	+ (1)
Basal cell papilloma	5	3	+ (3)	—	—
Basal cell carcinoma	9	3	+ (3)	—	—
Lichen planus	6	5	+ (5)	—	—
Contact dermatitis	5	2	+ (1)	—	+ (1)
Atopic dermatitis	4	—	—	—	—
Discoid lupus erythematosus	2	1	+ (1)	—	—
Psoriasis	3	1	+ (1)	—	—

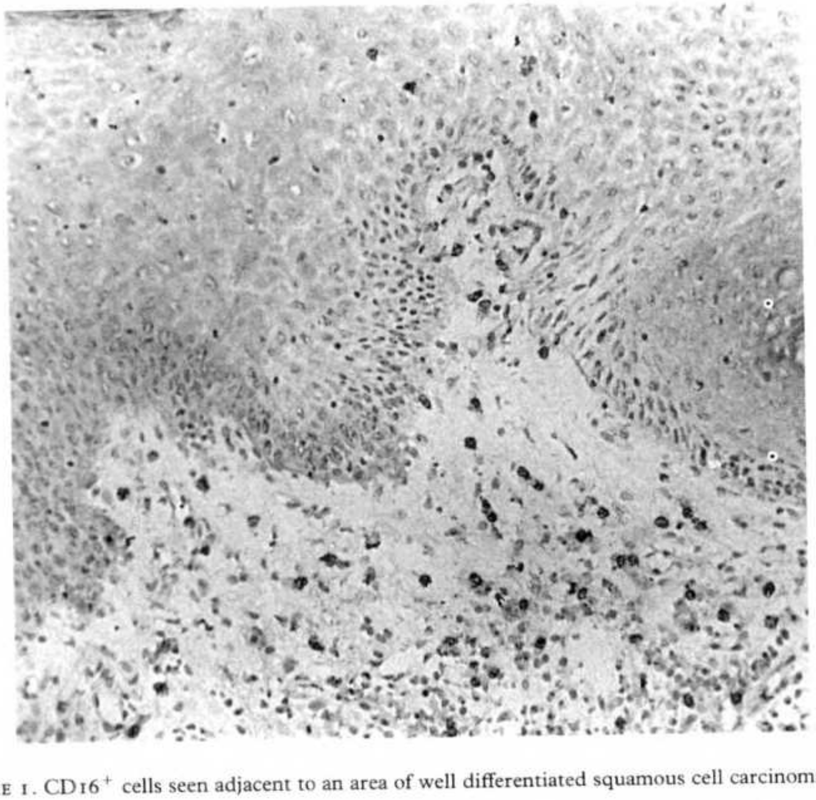

FIGURE 1. $CD16^+$ cells seen adjacent to an area of well differentiated squamous cell carcinoma. (× 57)

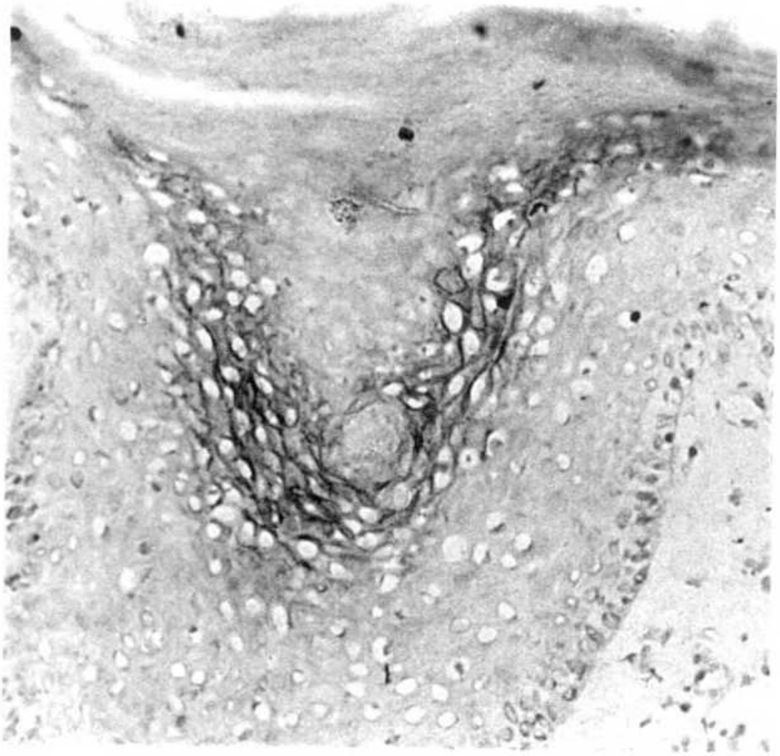

FIGURE 2. Leu 11 staining of subcorneal keratinocytes in the region of the acrosyringium. (× 90)

DISCUSSION

The infiltrate surrounding cutaneous tumours probably represents a specific host response. Analysis of such infiltrates and comparing results with those found in inflammatory dermatoses may clarify whether tumour specific responses exist. Although T lymphocytes occupy a central role in cell-mediated tumour cytotoxicity, nude mice deficient in cell-mediated immune mechanisms show an increased prevalence only of DNA virus-induced tumours,[20] whilst the beige mouse with an *in vitro* impairment of NK cell activity has a higher incidence of primary tumours.[21] These animal studies emphasize the possible importance of natural immunity mechanisms in human tumour defences.

Studies of cutaneous disease to date using HNK-1 antigen as a marker of NK cells have reported positive cells in melanoma and squamous cell carcinoma,[13,14] cutaneous allografts and amiodarone deposition,[19] basal cell carcinoma,[15] and lichen planus, contact dermatitis and cutaneous lymphoma.[16] Our study suggests that dermal HNK-1⁺ cells are a non-specific finding common to many inflammatory dermatoses and epidermal tumours. Serial sections in such lesions in our study reveal few HNK-1 cells carrying the NK specific CD16 and NKH-1 antigens. As the majority of cells in peripheral blood which demonstrate natural killer cell activity share the CD3⁻, CD16⁺, NKH-1⁺ phenotype, our findings imply that the majority of HNK-1⁺ cells in cutaneous tissue infiltrates are not NK cells, and more likely represent a cross-reaction of Leu 7 with T lymphocytes.

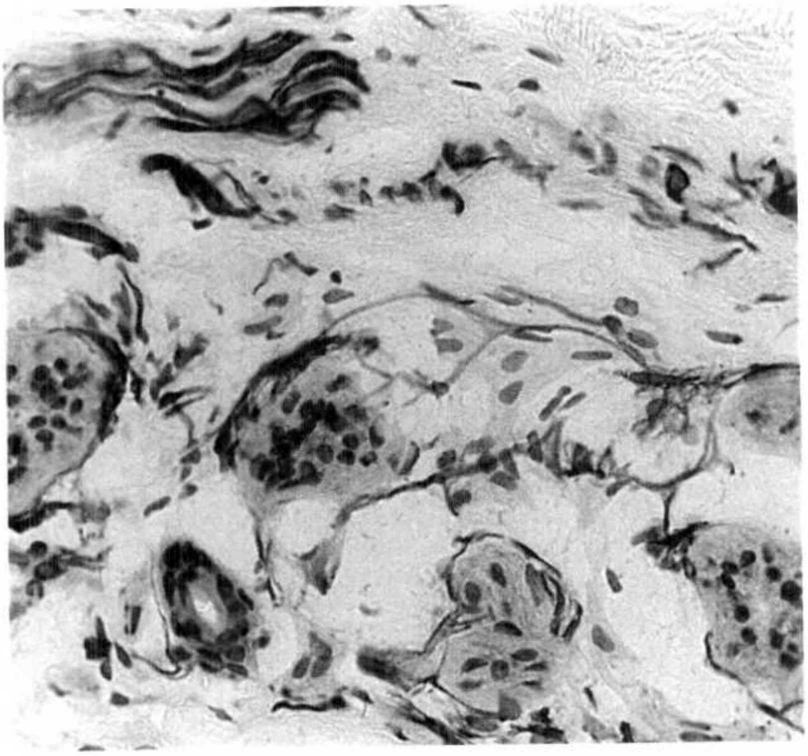

FIGURE 3. Leu 19 staining of cutaneous nerves around sweat ducts. (×225)

Cells carrying the more specific NK cell antigens CD16 and NKH-1 showed marked restriction in their tissue distribution. CD16$^+$ cells were restricted to the infiltrate surrounding squamous cell carcinoma and keratoacanthoma. Our findings suggest that not all these cells co-express NKH-1. These CD16$^+$/NKH-1$^-$ cells are mononuclear and do not represent Leu 11$^+$ neutrophils, and probably represent a true NK cell phenotype. Within NKH-1$^+$ cells, three distinct phenotypic populations have been identified,[10] one of which is CD 16$^-$. It is possible that these cells represent NK cells, or alternatively they may be CD3$^+$/NKH-1$^+$ non-MHC restricted CTL.[10]

As with CD16$^+$ the majority of NKH-1$^+$ cells were found in association with squamous cell carcinoma and keratoacanthoma. Such a distinct distribution of cells carrying CD16/NKH-1 antigens may suggest that NK cells are present around these squamoproliferative tumours to mediate a specific tumour immunity mechanism. However, in four of nine cases of SCC and four of seven cases of KA no CD16$^+$/NKH-1$^+$ cells were detected, in spite of examining multiple sections. Furthermore, the low percentage of positively stained cells (less than 5% of the inflammatory infiltrate) raises the possibility that NK cells may be present in insufficient numbers to produce effective natural immunity. The density of Leu 7 (not CD16/NKH-1) positive cells around malignant melanoma and squamous cell carcinoma was shown to be 10^2–10^4 times lower than that required for demonstrating natural killer cell activity in *in vitro* assays.[13] Although positive cells were present in small numbers in our study, the *in vivo* effector function of NK cells may be a qualitative rather than a quantitative phenomenon; it is established that individual NK cells may recycle and attack successive tumour cells over a prolonged period of time.[22]

We regard the HNK-1 antigen as being insufficiently specific to be used as a cutaneous marker for NK cells. Although the *in vitro* activity of NK cells has been defined, the biological *in situ* role of NK cells in anti-tumour responses is not yet established. The finding in this study of CD16⁺/NKH-1⁺ cells in the infiltrates around squamous cell carcinoma and keratoacanthoma is of interest, but our results cast doubt on a major role played by NK cells in these squamoproliferative tumours.

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Novel variants of human papillomavirus type 2 in warts from immunocompromised individuals

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SUMMARY

Twenty cutaneous warts from 18 renal transplant recipients (RTR) and a single wart sample from a patient suffering from Hodgkin's disease were screened for the presence of human papillomavirus (HPV) sequences by Southern blot analysis. Thirteen samples were shown to contain HPV DNA sequences, one was identified as HPV-3, two as HPV-4, six as HPV-2 and four were unidentifiable by restriction digest analysis. HPV-3 was identified in the wart sample from the patient suffering from Hodgkin's disease. In the six samples in which HPV-2 was found, the viral genome showed polymorphism for the PvuII and PstI sites. Two PvuII patterns and one PstI pattern are novel.

Patients subject to immunosuppression over long periods of time, such as organ transplant recipients, have an increased incidence of viral wart infections[1–4] with a greater propensity for malignant change.[5–7] The incidence of infection increases with length of time following transplantation.[6] Within a year of transplantation up to 42% of immunosuppressed patients have viral warts, and cutaneous squamous cell carcinomas are about 35 times more frequent than in the general population.[8] Epidermodysplasia verruciformis (EV) is a rare autosomal, recessively inherited disease in which there is a defect in immune function, multiple wart-like lesions are seen and malignant change has been observed in 25–30% of the cases.[9] Several HPV types have been found in viral wart-like lesions in patients with EV, and some are EV specific whilst others are also found occasionally in the general population.[10] Certain types, specifically HPV-5 and HPV-8, are more frequently associated with cancer[11–13] and this observation has led to the hypothesis of 'more oncogenic' HPV types.

Wart samples from renal transplant recipients (RTR) present an opportunity for studying the prevalence of HPV types in therapeutically immunosuppressed individuals. In this study we

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report the identification of several types and subtypes of HPVs from the biopsies of warts of renal transplant recipients.

METHODS

Twenty wart samples from 18 renal transplant recipients (RTR), treated with cyclosporin A or prednisolone with azathioprine, and a single wart from a patient with Hodgkin's disease, treated with prednisolone and radiotherapy, were studied. Eighteen of the RTR warts were unequivocal verruca vulgaris on the hands, arms and one sample from the thigh. One RTR sample was a filiform wart from the face and one was an actinic keratosis from the hand. A planar wart was obtained from the patient with Hodgkin's disease. Thirty wart samples were also

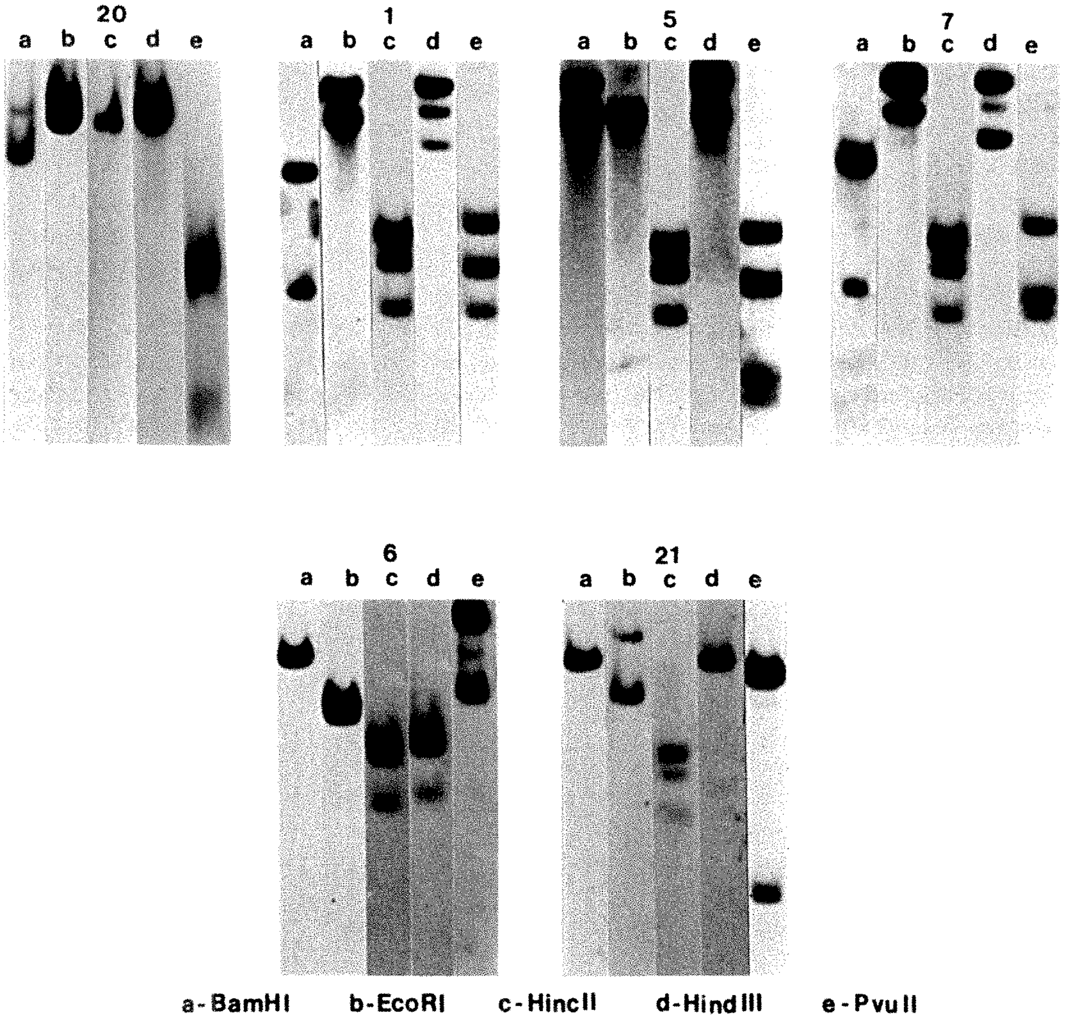

FIGURE 1. Detection of HPV DNA sequences in wart samples from immunocompromised patients. Tissue DNA (10 μg) was digested with (a) BamHI, (b) EcoRI, (c) HincII, (d) HindIII and (e) PvuII and after agarose gel electrophoresis and Southern blotting, was hybridized to mixed HPV-1 and 2, HPV-3 and 4, HPV-5 and 8 probes labelled with ^{32}P. The numbers refer to the samples.

obtained from clinically normal individuals. The warts were curetted off under local anaesthesia, and snap frozen in liquid nitrogen. The DNA was extracted and purified from the frozen material by the guanidinium thiocyanate procedure.[14] Routinely, 5–10 μg DNA was digested with restriction endonucleases according to the manufacturers' instructions (BRL, U.K. or Boehringer Mannheim, Middlesex, U.K.), electrophoresed in 1% vertical agarose gels, stained, photographed[15] and blotted onto Biodyne A nylon membrane.[16] HPV-1, -2, -3, -4, -5, and -8 DNA was excised from recombinant plasmids by digestion with appropriate restriction enzymes, labelled with ^{32}P by nick translation[17] and incubated with membranes overnight in 30% formamide, 5 × SSC at 42°C (SSC: 0·15 M sodium chloride and 0·015 M sodium citrate, pH 7·0). The membranes were then washed extensively in either 1 × SSC at 42°C with a final wash in 0·5 × SSC at 42°C or, for more stringent conditions, in 0·1 × SSC at 60°C. Autoradiography was carried out at −70°C for varying lengths of time.

RESULTS

Thirteen RTR samples were positive for HPV DNA sequences. In all cases the viral genome was episomal as shown by the presence of circular DNA in digests where the enzyme does not cut the viral sequence (Fig. 1, samples 20a; 1d; 5d; 7d; 6e and 21b). Six samples were positive for HPV-2, two for HPV-4, one for HPV-3 and four for viral sequences which could not be identified by restriction digest analysis.

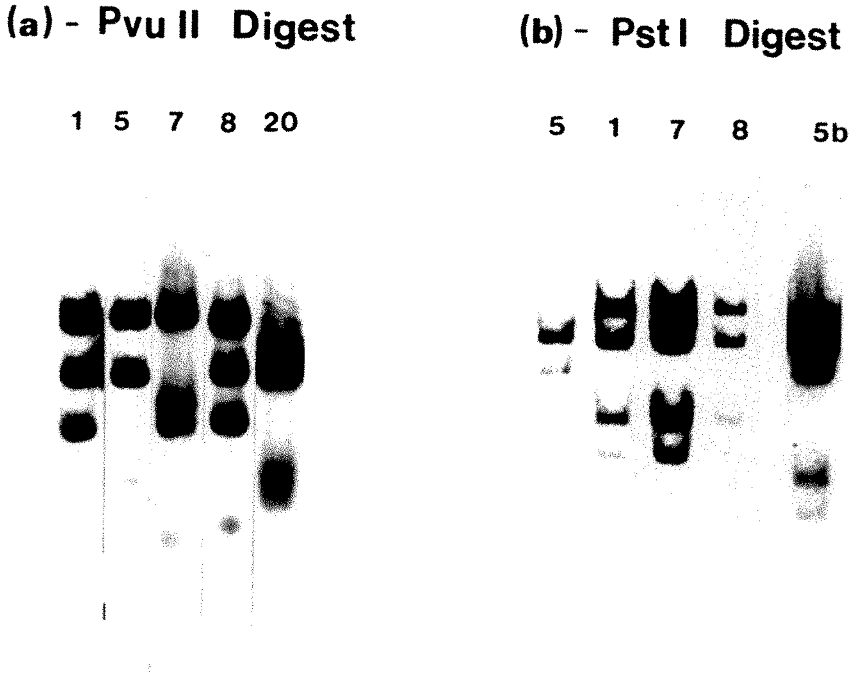

FIGURE 2. Southern blot analysis of (a) PvuII digest and (b) PstI digest of wart DNA samples (5 μg) positive for HPV-2. The probe was HPV-2 DNA labelled with ^{32}P. The numbers refer to the samples. In (b), lane 5b is a longer exposure of biopsy sample 5 showing more clearly the lowest molecular weight fragments.

TABLE 1. PvuII digest.

Fragment sizes (kbp)			
HPV-2a	Sample 5	HPV-2c	Sample 7
3·41	3·41	3·17	3·17
2·20	2·20	2·20	1·80
2·04	1·10	1·51	1·51
0·15	0·90	0·62	0·62
	0·15	0·30	0·40
			0·30

The 3·41 and 3·17 kbp fragments appear to co-migrate in the gel shown in Figure 2.

Six samples, 1, 4, 5, 7, 8 and 20, all from the RTR group, were positive for HPV-2. HPV-2b[18] was identified in sample 20 (Fig. 1) and HPV-2c[19] in samples 1, 4 and 8 (sample 1 is shown in Fig. 1). Digestion with PvuII gave rise to unexpected patterns in samples 5 and 7 (Figs 1 and 2a). The digest patterns obtained are variations which can be accounted for by the presence of extra PvuII sites (Table 1).

As de Villiers *et al.* reported a variant PstI digest pattern in HPV-2 DNA from a human tongue carcinoma,[20] samples positive for HPV-2 were digested with this enzyme. Samples 1, 7 and 8 gave rise to the expected pattern for HPV-2c[19] (Fig. 2b). However, sample 5 exhibited a pattern which differed from the published patterns, including HPV-2d (Michel Favre, personal communication; Table 2). We conclude that sample 7 contained a PvuII polymorphic variant of HPV-2c and that sample 5 contained a new subtype which we propose to designate HPV-2e.

Two RTR biopsy samples (6 and 14) contained HPV-4 DNA sequences[18] (sample 6 is shown in Fig. 1). HPV-4 has been found associated with squamous cell carcinoma of the skin[9] and of the oral cavity (A.Yeudall, personal communication), suggesting a possible casual role in cancer development.

TABLE 2. PstI digest.

Fragment sizes (kbp)			
HPV-2a	HPV-2b	HPV-2c	Sample 5
4·07	2·15	2·93	2·40 × 2
2·46	1·83	2·31	1·90
0·71	1·16	1·33	0·72
0·56	0·80	0·99	0·49
	0·73	0·23	0·23
	0·72		
	0·26		
	0·16		

Fragment sizes were estimated using HindIII digested Lambda DNA as molecular weight markers.

The wart sample from the patient with Hodgkin's disease (sample 21, Fig. 1) was shown to contain HPV-3a sequences.[21] Four samples were positive when probed under low stringency hybridization conditions with HPV-3 and HPV-4 DNA but not with other viral DNAs. The band patterns did not correspond to any of the known virus types and the hybridization signal was lost upon washing in stringent conditions. This suggests the presence of different virus types or subtypes related to HPV-3 and/or HPV-4. HPV DNA was not detected in eight of the RTR samples studied. This may be due to the absence of viral DNA,[22] or to the presence of viral types which do not cross-hybridize with the probes used.

Of the 30 cutaneous warts from normal individuals, 14 were positive for HPV DNA. HPV-2 was found most frequently, followed by HPV-1. No HPV-3 or HPV-4 DNA sequences were observed. Within the samples that were positive for HPV-2, four were HPV-2a, two HPV-2b and two HPV-2c. Two samples could not be subtyped with certainty because of limited material. However, no variant PvuII or PstI digest patterns were observed.

DISCUSSION

There was no obvious relationship between the type of immunosuppressive drug treatment and virus type detected. There was no correlation between different HPV-2 subtypes and premalignant or malignant conditions. No single wart contained more than one virus type, but different wart samples from one patient were found to harbour different virus types.

During the course of this work, two reports were published of HPV types found in warts from RTR.[23,24] These studies presented similar results to our own and in both, HPV-2 and HPV-4 were more frequently found in skin warts. HPV-1 and HPV-3 were infrequently found, and HPV-5 was identified in three benign lesions. Previously, HPV-5 had been identified in a benign lesion as well as in a skin cancer from the same renal transplant patient.[25] In our study HPV-5 was not found in any of our samples. In the previous studies restriction digest analysis was not detailed enough to identify subtypes of HPV-2, and therefore no variations were reported.

Although our study was conducted on a relatively small number of samples, our results were similar to other recent studies[23,24] and indicate that RTR are susceptible to multiple HPV infections with rarer types of HPV and with greater variation within HPV types. We also found that one of the warts (sample 5) contained a new subtype that we designated as HPV-2e.

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Human epidermal basal keratinocytes express CDw29 antigens

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SUMMARY

Two monoclonal antibodies, K20 and 4B4, assigned to the CDw29 cluster of differentiation antigens, were shown to react with basal keratinocytes (BK). The aim of this study was to identify the antigens recognized by K20 and 4B4 on epidermal cells, and to determine whether they were identical to those found on lymphocytes. Basal keratinocyte-enriched cell suspensions were labelled with ^{125}I and then 1% NP40 cell lysates were used for immunoprecipitation. Under reducing conditions, K20 and 4B4 immunoprecipitated from basal keratinocytes a broad MW 105 000 band and proteins of MW 145 000, 90 000 and 80 000. Under non-reducing conditions, each band was shifted down by approximately 5000 MW. Metabolic labelling studies demonstrated that the MW 145 000 and 105 000 subunits were synthesized by basal keratinocytes. On lymphoid cells, K20 and 4B4 are known to precipitate glycoprotein complexes made of a broad MW 130 000 protein band (β subunit) associated with a protein of MW 150 000 (α subunit) and proteins of MW 90 000 and 80 000 expressed in very low amounts. The MW 145 000 and 105 000 bands immunoprecipitated by K20 from basal keratinocytes correspond to the α and β subunits present on lymphoid cells. It has recently been demonstrated that K20 recognizes the common β subunit of the very late antigens family (VLA) and that 4B4 defines the helper–inducer subset of T lymphocytes. The present investigation provides evidence that basal keratinocytes share antigens of the VLA family with lymphoid cells and that they play an important role in the immune response in the skin-immune system.

The epidermis is composed mainly of keratinocytes, but among them are Langerhans cells (2–4%), melanocytes (1%) and a very small number of T lymphocytes. The epidermis is important for the initiation of immune responses and Langerhans cells that bind antigens within the skin provide an immunological surveillance.[1–3] The few intraepidermal T lymphocytes are of the CD8 suppressor-cytotoxic T-cell subset and are responsive to antigenically foreign epitopes.[4] In addition, the keratinocytes are able to assume a role in immunoregulation because

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of secretion of cytokines, particularly the IL-1 -like, epidermal cell-derived thymocyte activating factor (ETAF),[5,6] IL-3,[7] GM-CSF (identical to the keratinocyte-derived T-cell growth factor (KTGF)),[8] and soluble factors that inhibit lymphoproliferative responses (ELDIF, KLIF).[9,10] Keratinocytes can also be induced by γ-interferon to express the LFA-1 ligand, intercellular adhesion molecule-1 (ICAM-1), a surface molecule that regulates adhesion to T lymphoblasts and thereby participates in immune-mediated pathways.[11,12] Antigenic cross-reactivities and functional similarities between epidermis and thymus have been demonstrated[13-16] and several anti-leucocyte monoclonal antibodies (MAb) have been shown to cross-react with various skin components.[17]

Of the anti-leucocyte MAbs tested on human skin sections, 4B4 and K20, which are assigned to the CDw29 cluster of differentiation antigens, reacted with the membrane of basal keratinocytes (BK). The helper–inducer subset of $CD4^+$ cells is defined by 4B4[18] and K20 recognizes the common β subunit of the VLA family of adhesion molecules.[19,20] These MAbs are known to define surface glycoproteins preferentially expressed on lymphoid cells.

This study was undertaken to identify the antigens recognized by 4B4 and K20 on BK and to determine whether they are identical to the proteins found on lymphoid cells.

METHODS

Monoclonal antibodies

The MAbs K20, 4B4, and 2H4 were obtained through the exchanges of the Third International Workshop on Human Leukocyte Differentiation Antigens.

The MAb K20 was kindly provided by Dr Boumsell (INSERM U 93, Paris, France). In humans, K20 identifies glycoprotein complexes preferentially expressed on early haematopoietic cells, monocytes and a subset of peripheral blood T cells, and not on peripheral blood B cells. These glycoprotein complexes are composed of a constant MW 130000 β subunit non-covalently associated at the cell surface with a MW 150 000 α subunit and proteins of MW 90 000 and 80 000[21] (Amiot *et al.* in preparation).

The MAb 4B4 identifies a cell surface protein that is widely distributed throughout the haematopoietic system, and defines the human helper–inducer subset ($T4^+$ $4B4^+$).[18] 4B4 and K20 have been assigned to the CDw29 cluster of differentiation.[22]

The MAb 2H4 identifies a MW 220 000–200 000 surface glycoprotein which defines the human suppressor-inducer subset ($T4^+$ $2H4^+$).[23]

The MAb B-5G10 which specifically recognizes the $\alpha 4$ subunit of VLA-4, was a generous gift from M. Hemler (Dana Farber Cancer Institute, Boston, MA, U.S.A.).

Cells

Epidermal cell suspensions. Cell suspensions were obtained by trypsinization (0·2% trypsin for 18 h at 4°C) of human normal skin specimens. The epidermis was detached from the dermis with fine forceps and the epidermal sheets stirred in minimum essential medium (MEM, Gibco Grand Island, NY, U.S.A.) supplemented with 20% fetal calf serum (FCS). The resulting suspension was filtered through gauze and centrifuged for 10 min at 400 ***g***. Following a double wash in MEM-FCS, the cells were counted and their viability was assessed by trypan blue exclusion. The viability was 87% or higher.

This epidermal cell suspension was then selected for BK cells by the specific ability of these cells to adhere to collagen.[24] The cells were placed in type I collagen-coated Petri dishes (Bioetica, Lyon, France) for 1 h at 37°C and the firmly attached cells removed with 0·05%

trypsin with 0·02% EDTA in PBS for 5 min at 37°C. The cells were washed in MEM-FCS and the resultant cell viability was 95% or more.[25]

Lymphocytes. Human peripheral-blood mononuclear cells were isolated from healthy volunteer donors by Lymphoprep$^{\mathrm{TM}}$ density-gradient centrifugation (Nycomed AS, Oslo, Norway). Unfractionated mononuclear cells were depleted of adherent cells by adherence to plastic (1 h at 37°C).

Fluorescence-activated cell sorter analysis

Flow cytometric analysis was performed on a Cytofluorograph 50H (Ortho Instruments, Westwood, MA, U.S.A.). BK-enriched cell suspensions were incubated with 4B4 and K20 at a 1 : 200 dilution for 1 h at 4°C, followed by washings and labelling with FITC-conjugated F(ab′)$_2$ GaM IgG (Zymed, Burlingame, U.S.A.). Mouse myeloma UPC10 Ig2a and MOPC21 Ig1 (Sigma, St Louis, MO, U.S.A.) were used at the same dilution as negative isotype controls.

Radiolabelling and immunoprecipitation

Cells were externally labelled with ^{125}I using the glucose oxidase/lactoperoxidase technique.[26] Also, BK-enriched suspensions were metabolically labelled with ^{35}S-methionine (100 μCi/ml) for 18 h at 37°C. Labelled cells were extracted with Tris-buffered saline containing 1% NP40

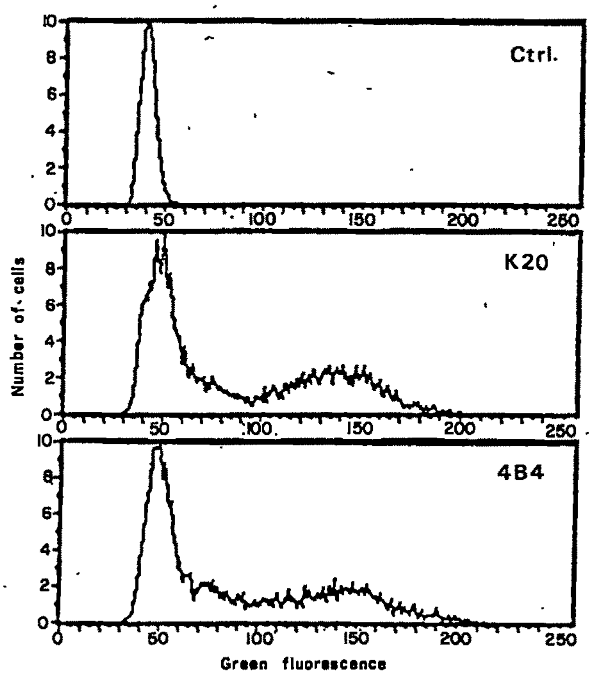

FIGURE 1. Cytofluorometric analysis of BK-enriched cell suspensions stained with K20 and 4B4. Distribution of cells is presented according to the number of cells (y-axis) and green fluorescence (x-axis). Histograms from K20- and 4B4-expressing BK are identical. No significant difference was observed between either the number of 4B4- and K20-positive cells (70 and 73% respectively—mean of three experiments), or the mean fluorescence channel (145 and 140 respectively). Ctrl, mouse IgG control.

and immunoprecipitation was carried out using a solid immunoisolation technique.[27] The immunoprecipitates were washed, resuspended in sample buffer (with or without 5% 2-mercaptoethanol), and applied to SDS-polyacrylamide slab gels.

Gel electrophoresis

Electrophoresis was performed both on a 7·5% acrylamide gel and on a linear gradient (5–10%) of acrylamide using Laemmli's method.[28] Molecular weight standards were ^{14}C-methylated proteins from Amersham.

RESULTS

By using the specific ability of BK to adhere to collagen, suspensions containing an average percentage of BK (80% ± 10%) were obtained, as checked by labelling with bullous pemphigoid serum.[25]

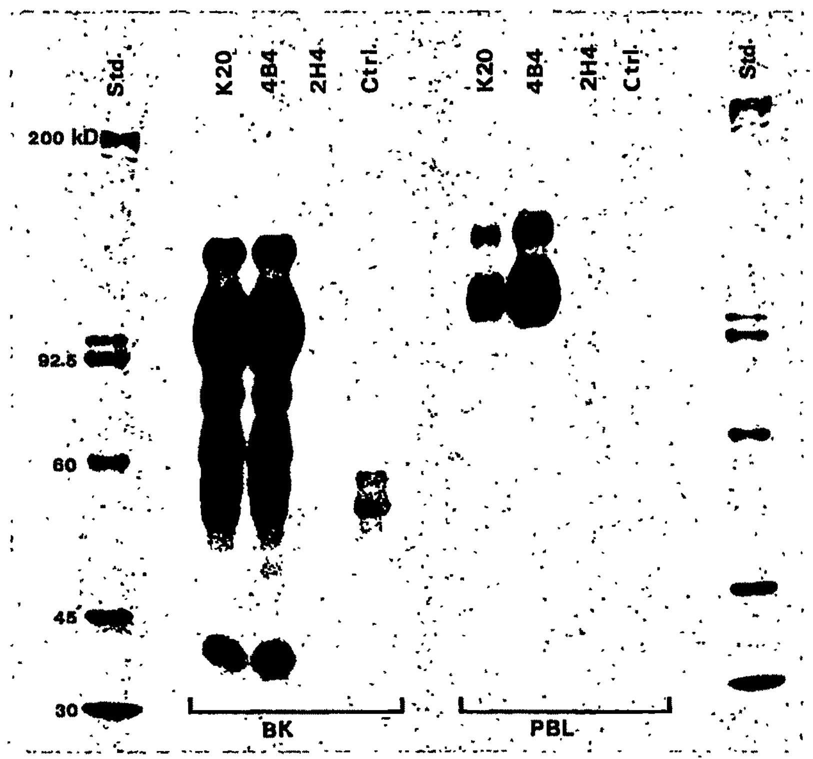

FIGURE 2. Comparison of the proteins recognized by K20, 4B4 and 2H4 on BK and peripheral blood lymphocytes (PBL). Gel analysis was performed on a linear gradient (5–10%) of acrylamide in the absence of 5% 2-mercaptoethanol. K20 and 4B4 immunoprecipitated a broad MW 100000 band (β subunit), a band of MW 140000 (α subunit) and proteins of MW 82000 and 70000 from BK. K20 and 4B4 immunoprecipitated a broad MW 120000 band (β subunit) and a protein of MW 140000 (α subunit) from PBL. Proteins of MW 82000 and 70000 are precipitated in very small quantities and are not visible on this fluorogram. Ctrl, mouse IgG control; two nonspecific background bands (MW < 60000) are visible. Standards (Sd) are myosin (200 kDa), phosphorylase b (92·5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa) and lysozyme (14·3 kDa).

Flow cytometric analysis (Fig. 1) showed that the 4B4- and K20-recognized proteins were both expressed by epidermal BK.

Under non-reducing conditions, K20 and 4B4 immunoprecipitated, from externally labelled BK-enriched suspensions, a broad MW 100 000 protein band and proteins of MW 140 000, 82 000 and 70 000 (Fig. 2). A band of approximately MW 40 000 was also precipitated from BK-enriched suspensions. The same pattern was obtained with both MAbs. Similar patterns were obtained when immunoprecipitations of ^{125}I-labelled lymphocytes were carried out, but protein bands of different relative mass were observed. K20 and 4B4 precipitated a broad MW 120 000 band (β subunit) and a protein of MW 140 000 (α subunit) (Fig. 2). The protein bands of MW 80 000 and 70 000 were immunoprecipitated in small quantities and were visible upon longer exposure time. These results are in accordance with those obtained when precipitation was carried out on lysates of various externally labelled leucocyte cells.[21] No bands were immunoprecipitated from BK and lymphocytes with the MAb 2H4. Controls were performed on the same gels (Fig. 2). In addition, no bands were immunoprecipitated from BK with MAbs reacting with the α and β subunits of LFA-1 (data not shown), demonstrating that this antigen was absent from BK and that no cross-reaction could be detected between members of the leucocyte integrin family and members of the VLA family via their β chain.

When immunoprecipitates were analysed using reducing conditions, both 4B4 and K20 antisera showed an increase of approximately 5000 MW in each protein band (results not shown). The lower mobilities of polypeptides observed in the absence of reduction are consistent with the presence of intrachain disulphide bridges within each unit.

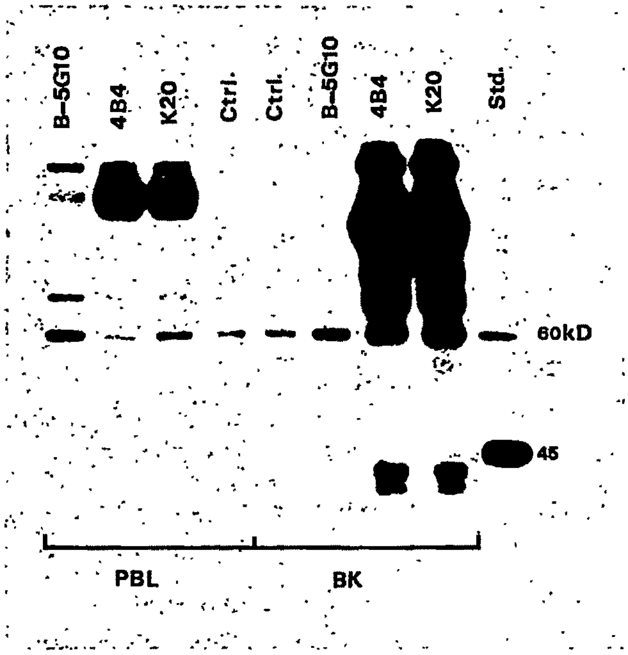

FIGURE 3. Comparison of the proteins recognized by K20, 4B4 and B-5G10 (anti-α VLA-4) on BK and PBL. B-5G10 immunoprecipitated the same protein complexes as K20 and 4B4 from PBL while no band was immunoprecipitated by B-5G10 (anti α VLA-4) from BK. Ctrl, mouse IgG control.

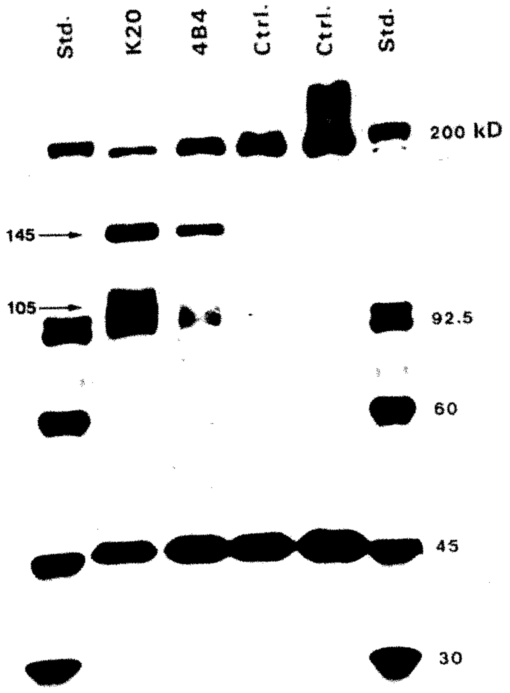

FIGURE 4. SDS-PAGE analysis of immunoprecipitate from lysate of ^{35}S-methionine labelled BK. BK were labelled for 18 h with ^{35}S-methionine. Gel analysis was performed in the presence of 5% 2-mercaptoethanol. The results show that the MW 145 000 and 105 000 bands were synthesized by BK. Control immunoprecipitations (Ctrl) with mouse IgG and negative MAb for BK are included to show background.

Similarly to the anti-α4 B-5G10 MAb, the 4B4 and K20 MAbs recognized the MW 150 000/130 000 VLA-4 complex on the surface of human lymphocytes as described by Hemler *et al.*[19] B-5G10 recognized relatively more α and less β subunit than was recognized by either 4B4 or K20. No bands were precipitated by B-5G10 from BK, indicating that the VLA proteins recognized by 4B4 and K20 on BK were different from VLA-4 (Fig. 3).

In order to determine whether the proteins precipitated by K20 and 4B4 were endogenously biosynthesized, immunoprecipitation of ^{35}S-methionine-labelled lysates obtained from overnight-labelled BK-enriched suspensions was performed. The results showed that the MW 145 000 and 105 000 protein bands were synthesized by these cells (Fig. 4).

DISCUSSION

Two CDw29 MAbs, 4B4 and K20, known to identify glycoproteins preferentially expressed on haematopoietic cells, monocytes and T cells, were found to react strongly with normal human epidermal basal keratinocytes. The specificity of these MAbs for a surface membrane antigen was demonstrated by double-indirect-immunofluorescence labelling and immuno-electron microscope studies.[29] In order to establish the identity of the antigens recognized by 4B4 and K20, immunoprecipitation analysis was performed using radio-iodinated membrane extracts of BK-enriched suspensions. 4B4 and K20 recognized (under reducing conditions) identical glycoprotein complexes composed of a prominent MW 105 000 subunit and proteins of MW 145 000, 90 000, and 80 000. These glycoprotein complexes are similar to those immunoprecipitated from normal human lymphocytes, with a slight difference in protein size. This difference could be due to variable patterns of glycosylation between cells of different lineages, as described for the MAb A-1A5 reactivity on certain non-lymphoid cell lines.[30] The MAb A-1A5 and the MAb K20 have recently been shown to be identical and are known to recognize the common β subunit of the VLA proteins.[19,20]

The human VLA protein family consists of six cell-surface heterodimers, each with unique α subunits that are non-covalently associated with a common β subunit.[19,20,30] These structures appear to have cell-matrix adhesion functions since VLA-2 has been identified as a collagen receptor, VLA-5 as a fibronectin receptor, and VLA-3 appears to have multiple ligands (collagen, fibronectin, laminin).[20] The VLA proteins belong to the integrin superfamily of cell adhesion molecules. This family of cell surface receptors is subdivided in three different families which are defined by their distinct β subunits.[31] The β1 family includes the human and the chicken fibronectin receptors and the VLA proteins. The β2 family, also called the leucocyte integrin family, includes three glycoproteins (LFA-1, Mac-1, p150–95) and the β3 subgroup is composed of the vitronectin receptor and the protein IIb/IIIa of platelets.[31] The cell surface distribution of the receptors of the β2 and β3 subgroups is restricted but nearly all tissues and cell types express one or more VLA complexes.[32] Our results are in favour of the presence of adhesive protein receptors of the VLA family on basal keratinocytes (α subunit of 145 000 MW and β subunit of 105 000 MW). Our data show that the VLA proteins recognized by 4B4 and K20 on BK are different from VLA-4, which is consistent with the observation that the VLA-4 structure is absent from, or only weakly present in most adherent cell lines.[19] The VLA proteins present on epidermal cells remain to be determined.

The expression of VLA receptor on BK and its absence on differentiated spinous and granular keratinocytes suggests the presence of a fibronectin receptor (FNR). It is known that FNR structures play a role in cell differentiation and maturation, and that these receptors are lost when the cells become more differentiated.[20] FNR structures could facilitate the anchoring of BK until they are ready to emigrate and then, once differentiated, they lose their adhesion capability. Multiple-related but distinct FNRs are known[20,33] and recently two cell adhesion receptors for fibronectin (ECMR I) and collagen (ECMR II), related to VLA-3 and VLA-2, have been described.[33,34] The precipitation by 4B4 and K20 of an additional MW 40 000 protein suggests that adhesion receptors for collagen may also be present on BK, since membrane glycoproteins with a molecular mass ranging from 35 000 to 45 000 (ECMR IV) which bind to both type I and VI collagen have been described.[33] Recently, immunohistochemical studies have shown that anti-α1- and anti-α4-VLA did not stain BK but anti-α2-VLA did.[35] Studies are in progress to try to identify the VLA structure(s) implicated in cell-matrix adhesion evidenced on BK.

The immunological role of the protein complexes recognized by K20 and 4B4 has been demonstrated. K20 inhibits CD3-induced T-cell activation and the monocyte structure recognized by K20 is directly required for this activation (Amiot *et al.*, in preparation). 4B4 defines the human helper-inducer subset of human peripheral blood T cells. Both MAbs are assigned to the CDw29 cluster of differentiation antigens and recently the CDw29 positive cells have been more generally designated as memory T cells.[36] These cells have the ability to proliferate vigorously when restimulated with an antigen to which the donor has been previously primed *in vivo*. The memory cells or CDw29-high subset are immunophenotypically distinguished by three markers: CDw29, LFA-3 and UCHL1. It is striking to note that BK express and synthesize CDw29 antigens,[37] (our data) and also express LFA-3.[38] This new indication of antigens shared between lymphocytes and keratinocytes is further evidence for the indirect role of BK in the immune response besides the production of various cytokines. Leucocytes have multiple adhesive receptors which mediate cell–cell interactions,[20] and the cell-matrix adhesion receptors of the VLA family expressed by lymphocytes and BK may be implicated in the interaction of mature, active lymphocytes with the extracellular matrix components of extravascular tissues such as the epidermis. The skin is exposed to exogenous antigens, and it is possible that BK participate in host defense by expressing 'recognition' molecules that play a role in the traffic and the homing of memory cells to the epidermis.

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Stratum corneum hydration and amino acid content in xerotic skin

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SUMMARY

The relationship between clinical severity, the hydration state of the skin surface as assessed by a conductance with a 3·5 MHz high frequency impedance meter, and the amino acid content of the stratum corneum (SC) of six patients with ichthyosis vulgaris and 30 elderly persons with varying degrees of xerosis was investigated. With an increase in the severity of xerosis the SC showed a decrease in hydration and in its extractable amino acid content. There was a significant correlation between the hydration state and amino acid content of the SC. Although there was a significant correlation between the amino acid content of the lower leg SC and that of the forearm SC in the same subject, the former was generally lower, corresponding to the greater incidence of xerosis on the lower leg. These results suggest that a decreased amount of amino acids may be the result of low profilaggrin biosynthesis in the epidermis and that this is involved in the pathogenesis of these xerotic skin conditions. Clinical improvement of the xerosis following treatment with a urea-containing cream was not accompanied by any significant change in the amino acid content of the SC.

Water-soluble substances of the stratum corneum (SC) play an important role in providing its hygroscopic properties.[1] The major components are amino acids and metabolites of amino acids such as pyroglutamic acid, which are produced mostly by the degradation of keratohyalin protein, i.e. histidine-rich protein or profilaggrin.[2,3] Ichthyosis vulgaris is an inherited disorder of keratinization in which there is a decrease or absence of profilaggrin, filaggrin[4] and keratohyalin granules.[5] Senile xerosis is commonly found in the elderly, especially in the winter.

So far there have been no quantitative studies on the relationship between xerosis and keratohyalin-derived natural moisturizing agents of the SC in different disorders of keratinization. We have studied the relationships between the severity of dry and scaly skin disorders with the hydration state and amino acid content of the SC, and in particular how the amount of

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natural moisturizing agents derived from keratohyalin proteins are related to the severity of xerosis. Also, after treatment with a topical preparation containing 10% urea, the changes induced in the amino acid content of the SC were determined.

METHODS

Subjects

The extensor surfaces of the lower leg of six patients with ichthyosis vulgaris and 30 elderly patients (age range 59–94 years) having varying degrees of dry skin ranging from normal to very dry were studied. Senile xerosis was graded as follows: grade 0, no change ($n=7$); 1, mild ($n=10$); 2, moderate ($n=8$); 3, severe ($n=5$). All the studies were carried out in the winter in Sendai.

Measurement of skin surface hydration state

The hydration of the SC was estimated by measuring skin surface conductance using a 3·5 MHz high frequency impedance meter (IBS Inc, Hamamatsu, Japan) equipped with central and peripheral electrodes of 2 and 4 mm in diameter.[6] The measurements were performed at ambient temperatures of 18–22°C and 40–50% relative humidity.

Determination of amino acid content of the SC

Surface samples of SC were obtained by a serial adhesive tape stripping. The SC samples were separated from the adhesive tapes by washing in toluene and then dried. The SC samples were homogenized in 0·3 N $HClO_4$ to extract the amino acids without solubilization of the proteins.

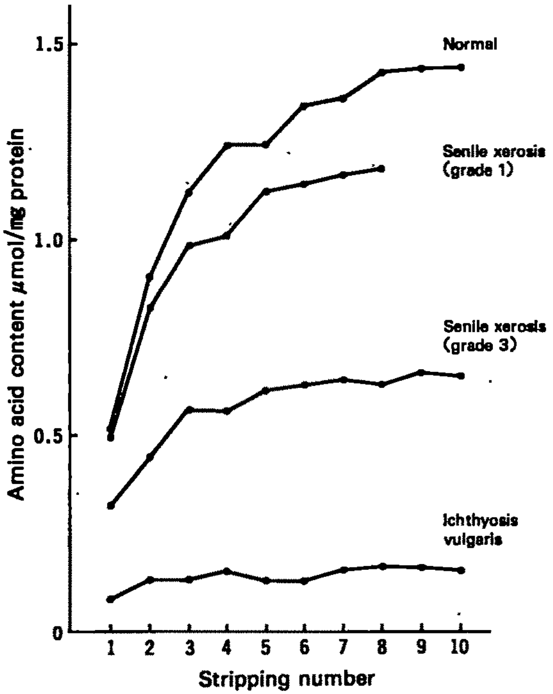

FIGURE 1. Amino acid content of serially stripped stratum corneum from the skin surface.

The homogenate was centrifuged and the amino acid content in the supernatant determined using an amino acid analyzer (Hitachi model 835) after neutralization with 0·3 N NaOH. The acid-insoluble precipitate was dissolved in 1 N NaOH, and the amount of protein was determined.[7] The same procedure was carried out with adhesive tape alone as a control which yielded only a negligible amount of protein. The amino acid content was expressed as nmol/mg of alkaline soluble protein. In addition to the SC samples from the lower legs, samples were also collected from less dry areas on the flexor surface of the forearms of the elderly patients.

Topical treatment with a urea-containing cream

Five patients with icthyosis vulgaris and 10 patients with senile xerosis (grade 1–3) were treated with a topical application of 10% urea-containing cream (Ferzea®, Shiseido Co Ltd, Tokyo, Japan) daily for 2–4 weeks. Before and after the treatment, clinical assessment of skin condition and determination of amino acid content of SC were performed.

Statistics

The statistical significance of the results was evaluated using Student's *t*-test.

RESULTS

Amino acid content in serially stripped SC

In one patient with ichthyosis vulgaris and three individuals with normal or dry skin the amino acid content of each of the serially stripped SC samples was determined (Fig. 1). The amino acid content in ichthyosis vulgaris was extremely low and showed little difference in the serially stripped samples. The amino acid content of the SC showed a gradual increase in the aged skin with the number of strippings and reached a plateau after the fifth stripping. There was a marked variation in the amino acid content between individuals with different degrees of xerotic change.

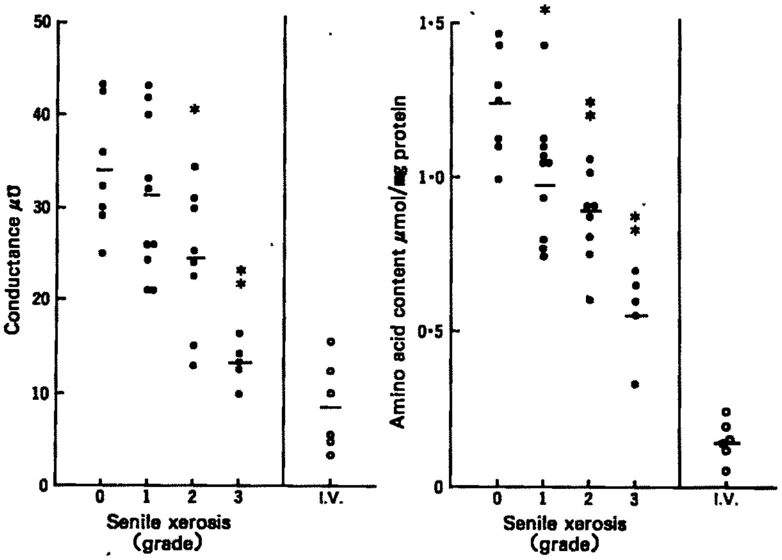

FIGURE 2. Relationship between skin surface conductance and amino acid content of stratum corneum of senile xerotic skin and ichthyosis vulgaris. $^{*}P<0·05$, $^{**}P<0·01$ as compared with grade 0.

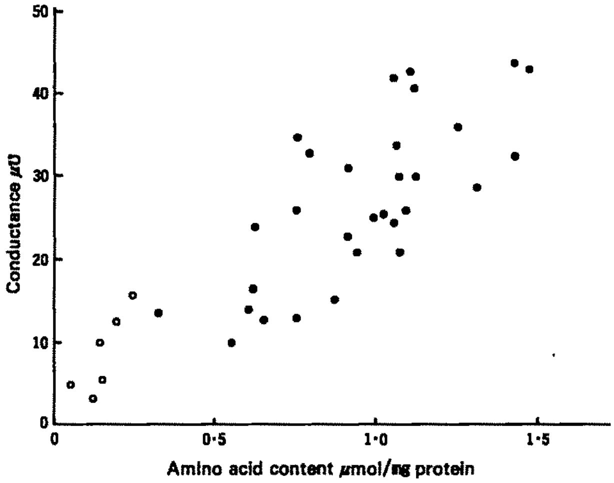

FIGURE 3. Relationship between amino acid content of the stratum corneum and skin surface conductance in patients with senile xerosis and those with ichthyosis vulgaris. ●, aged skin; ○, ichthyosis vulgaris. ($r = 0·81$).

Relationships between clinical features, skin surface hydration state and amino acid content of SC
Skin surface hydration state and amino acid content in the SC were compared in elderly persons showing different degrees of severity of xerotic skin and including those where skin appeared normal (grade 0). The hydration state and amino acid content of SC showed a significant decrease with an increase in the severity of the disorders (Fig. 2). All the patients with ichthyosis vulgaris examined in this study were classified as severe, and their skin surface hydration state

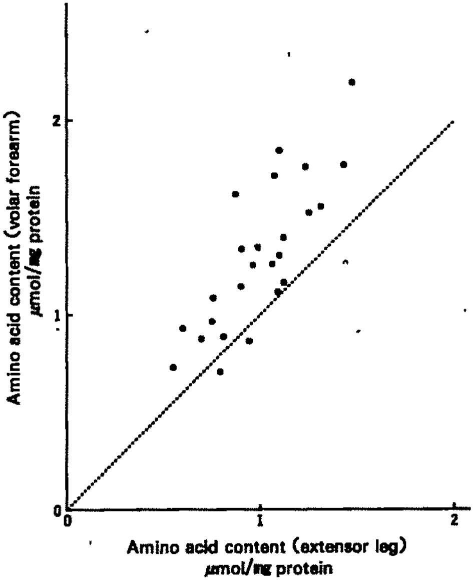

FIGURE 4. Relationship between amino acid content of the stratum corneum of the extensor surface of the lower legs and that of the volar aspect of the forearm in elderly subjects with xerosis ($r = 0·81$).

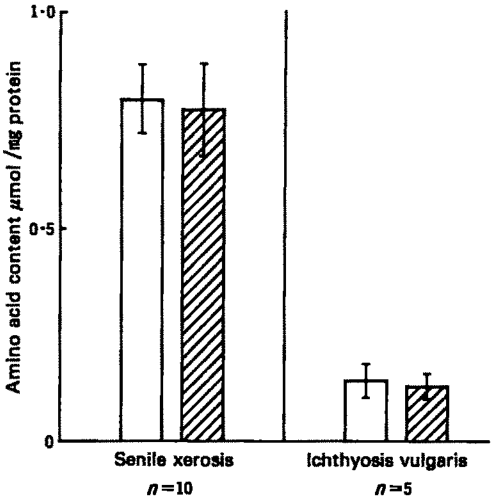

FIGURE 5. Effect of the treatment of a urea-containing cream on amino acid content of the stratum corneum in patients with senile xerosis and those with ichthyosis vulgaris. □, pre-treatment; ▨, post-treatment.

and amino acid content were extremely low (Fig. 2). A significant positive correlation was found between skin surface hydration state and amino acid content of the SC ($r = 0·81$, $P < 0·01$; Fig. 3). Although the skin of the flexor aspect of the forearm was not affected or only mildly so in the elderly individuals with xerosis, a significant positive correlation was also found between the amino acid content of the lower leg and that of the forearm ($r = 0·81$, $P < 0·01$; Fig. 4). The former was lower than the latter in most cases.

Effect of treatment with a urea-containing cream on the amino acid content of SC

A reduction of the chapping and scaling of the skin was observed in all patients with ichthyosis vulgaris and senile xerosis treated with the urea-containing cream. The clinical assessment showed a significant improvement in grade, from an average of 2·3 to 0·2 for senile xerosis and from 3·0 to 1·2 for ichthyosis vulgaris. However, no significant change was found in the amino acid content of the SC (Fig. 5).

DISCUSSION

In this study the amino acid content of tape strippings of the SC was measured. The values obtained appear to be similar to those of deeper layers of the SC (Fig. 1) and may be a good index of the synthesis and/or degradation of precursor proteins that were originally formed in the granular layer. The low levels of amino acids found in the SC of ichthyosis vulgaris patients corresponds with the finding of a decrease or absence of profilaggrin.[4] Our results also showed that amino acid content decreased in senile xerosis in relation to clinical severity and correlated with the diminution of keratohyalin granules in the xerotic skin lesions.[5] As we described,[3] the composition of free amino acids was similar to that of the precursor protein filaggrin, even in xerotic skin (data not shown).

In this study the amino acid content and the hydration state of the SC of the dry skin were related. This suggests that the amount of these amino acids, and perhaps the synthesis of their precursor proteins, play an essential role in determining the hygroscopic properties of the SC.

The amino acid content of the SC differed greatly in the subjects both on the lower legs and forearms. However, we found a significant correlation between the values for the two regions in the same subject. Such results suggest that the amount of amino acids and precursor proteins might be inherent for each individual, and an important factor in the propensity to develop xerotic skin. The lower incidence of dry skin on the forearm compared to the lower leg may be explained by the production of larger amounts of amino acids.

Treatment with the urea-containing cream did not alter values of the amino acid content of the SC in either ichthyosis vulgaris or senile xerosis, although it produced a marked clinical improvement. The urea preparation was effective in improving the defective water-holding ability of the SC and it is unlikely to have had any influence on keratinization.

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Large cell anaplastic lymphoma of the skin

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SUMMARY

Large cell anaplastic lymphoma (LCAL) is a recently described entity recognized by its constant expression of the CD30 antigen and by its morphology. LCAL is classified as high grade malignant lymphoma. We report 10 patients with LCAL of the skin, three with primary LCAL and others in which it developed with cutaneous T-cell lymphoma, mainly mycosis fungoides. The immunophenotype in all but one of these cases, was that of activated T cells.

Recently, the monoclonal antibodies Ki1 and HSR1 have been produced that recognize an antigen present on Hodgkin's lymphoma cell lines,[1–4] Hodgkin and Sternberg–Reed cells in Hodgkin's disease[2,3] and some B- and T-cell lymphomas.[5] This activation-antigen is present on phytohaemagglutinin activated T cells, virus-transformed lymphoblastoid cell lines and on macrophages,[6,7] and is designated as the CD30 antigen.[8] However, this antigen is not restricted to haematopoietic tissue, and is regularly expressed by tumour cells of embryonal carcinoma.[9] It is present on two membrane-bound proteins with molecular weights of 105 and 120 kDa.[10]

A group of large cell anaplastic lymphomas (LCAL) of T- or B-cell origin and occasionally a histiocytic phenotype have been shown to express the CD30 antigen.[5] These cases have been previously classified as malignant histiocytosis or even as undifferentiated carcinomas. LCAL is now included in the classification of lymphomas,[11,12] and in the more recent Kiel classification was designated as a high-grade malignant lymphoma. The diagnosis can be made by routine histology and LCAL appears to be a distinct clinical entity as regards its age of onset, distribution and biological behaviour. Although LCAL was first described with the unique expression of the CD30 (Ki1) antigen,[5] the diagnosis is now based on morphological grounds as CD30 expression is found in a variety of malignant lymphomas. LCAL has been previously reported to occur in the skin,[13–16] but detailed morphological and immunohistological studies have not been carried out. We report 10 cases of cutaneous LCAL, and differentiate these from other types of cutaneous T-cell lymphomas that show expression of the CD30 antigen.

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TABLE 1. Summary of cases

Number	Diagnosis	Sex	Age	CD5	CD3	CD2	CD4	CD8	CD7	Ki67	CD25	HLA-DR/DQ	CD30
1	LCAL prim.	m	57	+	ND	+	+	–	ND	70	ND	+	+
2	LCAL prim.	m	51	–	–	–	–	–	–	40	+	+	+
3	LCAL prim.	m	38	–	+	+	–	–	–	60	–	+	+
4	LCAL sec.	m	60	+	+	+	+	–	ND	40	ND	+	+
5	LCAL sec.	f	64	+	+	+	+	–	–	40	30	+	60
6	LCAL sec.	f	47	–	+	–	+	–	–	40	–	–	+
7	LCAL sec.	m	68	–	ND	+	+	–	ND	70	ND	+	+
8	LCAL sec.	m	93	–	ND	–	–	–	–	40	–	+	+
9	LCAL sec.	f	72	+	–	+	+	–	–	30	+	+	+
10	LCAL sec.	f	84	–	ND	–	+	–	–	40	+	+	90

prim., primary.
sec., secondary.
ND, not determined.

TABLE 2. Designation and specificity and source of monoclonal antibodies used in present study

Cluster	Monoclonal antibody	Source	Specificity
CD1	Leu 6	Becton–Dickinson(BD)	Thymic cortex cells, Langerhans cells, interdigitating reticulum cells
CD2	Leu 5	BD	T cells
CD3	Leu 4	BD	T cells
CD5	Leu 1	BD	T cells
CD4	Leu 3	BD	Helper/inducer T cells, macrophages
CD8	Leu 2	BD	Suppressor/cytotoxic T cells
	OKT 9	Ortho Diagnostic	Transferrin receptor (TR)
	Leu 8	BD	T-cell subset, follicle mantle cells
CD25	α-Tac*		Interleukin-2 receptor
CD30	Ki1†		Activation antigen of lymphatic and histiocytic cells
	Ki67†		Proliferating cells
CD22	HD39‡		B cells
HLA-DR/DQ	TÜ35§		HLA class II
HLA-DQ	Leu10	BD	HLA class II

Monoclonal antibodies kindly provided by *Th.Goldmann, †H.Lemke, ‡L.B.D.Dörken, §A.Ziegler.
Becton Dickinson, Rodermark, F.R.G.
Ortho Diagnostic System, Raritan, NJ, U.S.A.

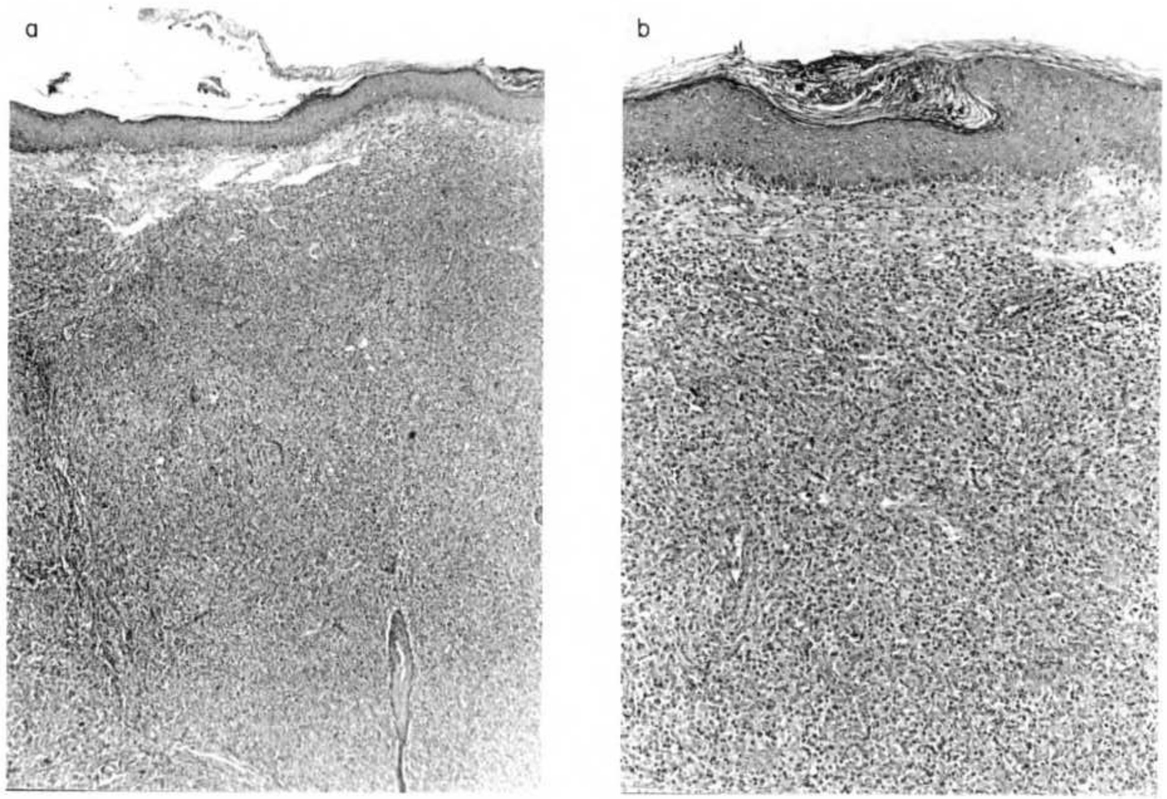

FIGURE 1. (a) Secondary LCAL of the skin, arising in a patient with long standing mycosis fungoides (case 5). Free subepidermal grenz zone; diffuse infiltration of the entire dermis. For cellular details see Figures 1b and 3. (b) Higher magnification of Fig 1a (case 5). Note the cohesive growth pattern.

METHODS

Ten cases of LCAL primary to the skin were investigated (six male, four female; mean age, 60 years). All expressed the CD30 antigen (Table 1). In four of the cases there was lymph node involvement.

Biopsies were taken from all the patients and divided into two portions. One was fixed in formaldehyde and embedded in paraffin; 5 μm sections were cut and stained with haematoxylin and eosin, Giemsa and periodic acid Schiff. The other portion was snap frozen in liquid nitrogen, and 5 μm cryostat sections were cut and fixed in acetone for later immunostaining using an immunoperoxidase[17] or APAAP[18] method. The monoclonal antibodies used for immunophenotyping are listed in Table 2.

RESULTS

Clinical findings

Five out of 10 cases of LCAL developed in patients with mycosis fungoides and two had a pleomorphic T-cell lymphoma. Three cases were diagnosed as primary LCAL. The data concerning age and sex of the affected patients are summarized in Table 1. As most of the cases were sent to us from other institutions, the data concerning their clinical course were not available.

Morphology

All the cases showed a diffuse infiltrate in the upper and lower dermis (Figs 1a, b), and in three there was involvement of the subcutaneous fat. The infiltrate consisted of sheets of tumour cells

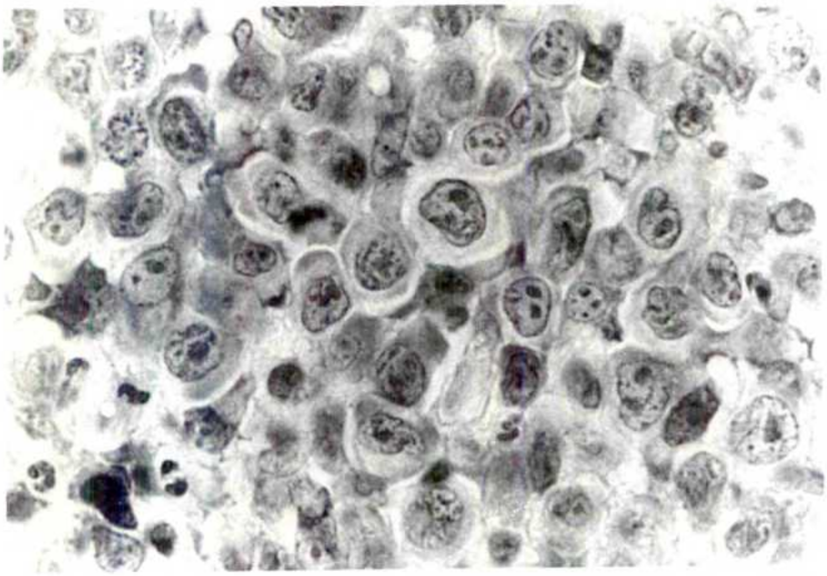

FIGURE 2. Primary LCAL of the skin. High power magnification showing the characteristic large pale cells with abundant clear cytoplasm, distinct nuclear membrane and prominent nucleoli.

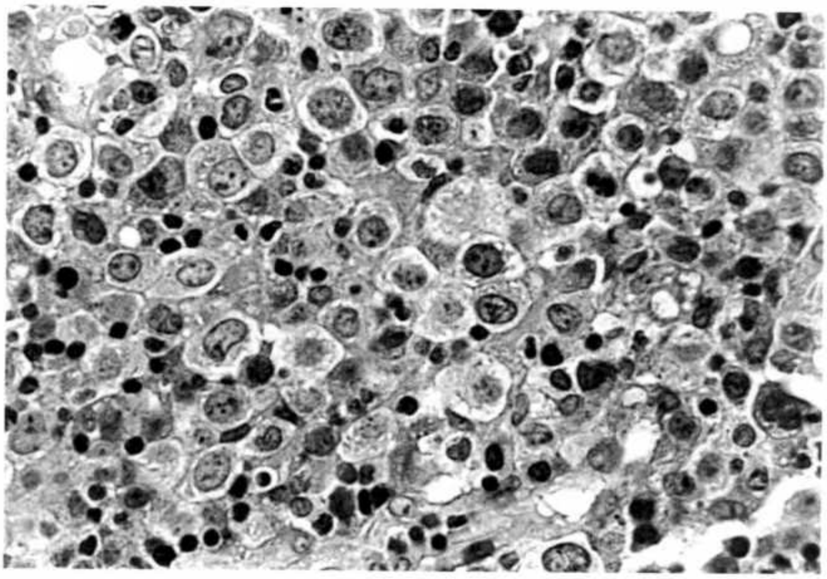

FIGURE 3. Secondary LCAL of the skin. High power magnification with large cells and between them small to medium, pleomorphic cells.

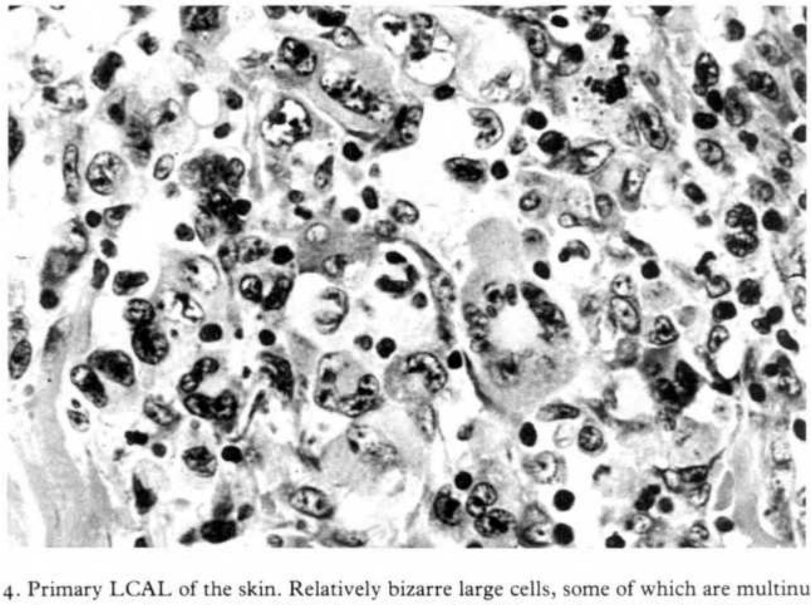

FIGURE 4. Primary LCAL of the skin. Relatively bizarre large cells, some of which are multinucleated. Small lymphocytes are intermingled. This bizarre type of LCAL has often been classified as 'malignant histiocytosis'.

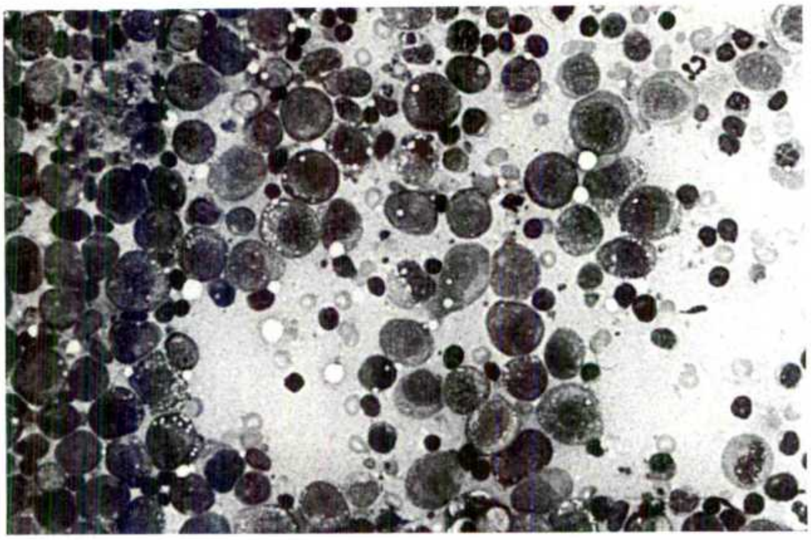

FIGURE 5. LCAL of the skin; imprint. Admixture of large tumour cells with small and medium sized ones. Note vacuoles of large tumour cells.

sometimes with small foci of necrosis. There was no epidermotropism of the cells. The tumour cells were characterized by distinct cell and nuclear membranes and had large round or oval nuclei, with one or two prominent nucleoli and dense chromatin around the nuclear membrane (Figs 2 and 3). Some giant cells were always present and in two cases the infiltrate was dominated by these cells, most of which resembled Hodgkin or Sternberg–Reed cells (Fig. 4). In imprints, cytoplasmic vacuoles were frequently found in these cells (Fig. 5). Neutrophils and more frequently eosinophils were found in the infiltrate and there were many histiocytes. In secondary LCAL, some medium and small sized lymphocytes were usually found between the large tumour cells and they exhibited marked nuclear pleomorphism (Fig. 3).

Immunohistochemistry

The immunohistochemical findings are summarized in Table 1. Cases classified as primary (unrelated to any preceding lymphoma) or secondary (arising in another cutaneous T-cell lymphoma) LCAL expressed the CD4 antigen in seven of the cases. Four of the cases lacked the sheep erythrocyte receptor (CD2) and two out of six were negative for CD3. Only four cases expressed the CD5 antigen and none expressed the CD7 or CD8 antigen. In all cases the tumour cells were positive for CD30 (60– > 90%) and apart from one had HLA-class II antigens on their cell surface. The expression of IL-2-receptors (CD25) was found on the tumour cells in three out of seven cases, and one was partly CD25 positive.

Within the LCAL infiltrates, only a small number of $CD1a^+$ cells were found in three of the cases, and these were absent in the others. The percentage of Ki67 (proliferation associated antigen) positive cells was 49 ± 16% in primary and secondary LCAL.

DISCUSSION

It is now generally accepted that the diagnosis of LCAL can be made solely on morphological grounds. The lymphoma has large cells with abundant clear cytoplasm that appears slightly asophilic or grey with Giemsa stain. The cells have round or oval and sometimes irregularly haped nuclei with a distinct membrane that may show pleomorphism and there may be many typical mitoses. The cells frequently have a cohesive growth pattern and bizarre giant cells esembling Hodgkin or Sternberg–Reed cells are found. In lymph nodes the lymphoma requently involves the sinus areas in the early stages. In the skin, LCAL presents with nodular r diffuse infiltrates in the dermis and only rarely invades the epidermis, though involvement of he subcutaneous fat may occur.

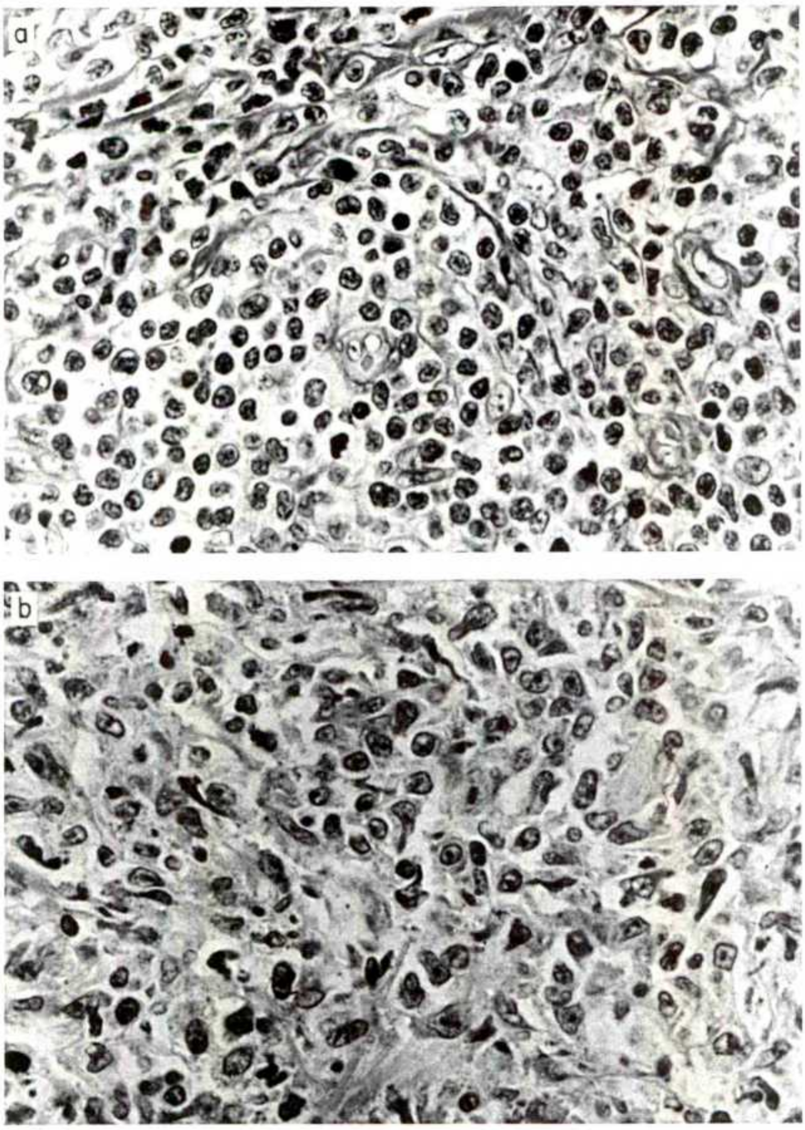

FIGURE 6.(a) Pleomorphic T-cell lymphoma of the skin, small and medium cell type. Tumour cells show considerable variation of their nuclear shape but similar size. (b) Pleomorphic T-cell lymphoma of the skin, large cell type. Blast-like cells with large cytoplasm and variable nuclear contours.

CALCUTTA CENTRAL

Other cutaneous lymphomas and related disorders have to be considered in the differential diagnosis, i.e. pleomorphic T-cell lymphoma (PTCL) of the large cell type, and lymphomatoid papulosis (LP).[11,12] It is not difficult to separate small and medium sized PTCL (Fig. 6a), while the infiltrate in large-cell PTCL (Fig. 6b) is not as homogeneous as that found in LCAL.[11,12] LP can be separated from LCAL by the perivascular arrangement of the infiltrate, that is often pleomorphic and contains small lymphocytes, eosinophils and neutrophils. There is however, an overlap between these two entities especially when, in LP, there are large sheets of anaplastic cells.

So far, including our own series, there are 25 reported cases of LCAL occurring in the skin.[13–16,19] As shown in Table 3, 18 out of 25 cases were of T-cell origin of an activated helper T-cell phenotype, and one was of a B-cell lineage. A similar predominance of T-cell phenotype was also found in lymph node LCAL.[5] The remaining cases were childhood Ki1 lymphomas. The term Ki1 lymphoma used in previous publications should be discarded because Ki1 designates a widespread antigen rather than a distinct clinicopathological entity.

The childhood LCAL with lymph node and skin involvement is defined by the lack of T-cell antigens but expresses CD25, transferrin receptor and CD30. These cases that formerly had been classified as regressing atypical histiocytosis or related histiocytic syndromes, clearly represent LCAL and are identical to the cases described in this paper.[20–22] Despite the lack of T-cell surface antigens, rearrangement of the β-chain gene of the T-cell receptor was demonstrated in one case, thus implying T-cell lineage.[19]

All the reported cases of cutaneous LCAL, including our own, were over 40 years of age. The

TABLE 3. Immunophenotypical profile of different CD30 positive T-cell lymphomas and related disorders of the skin

	Cases	CD1	CD2	CD5	CD3	CD4	CD8	CD7	TR	CD25	DR	Ref.
LCAL	5	–	+	4/5	+	3/4	–	ND	+	+	+	11
	1 B	–	–	–	–	–	–	–	+	+	+	11
	3	ND	+	+	2/3	+	–	ND	ND	2/3	+	4
	6*	–	–	–	–	–	–	ND	+	+	+	19
LP	9	–	+	6/9	+	+	–	ND	3/9	+	+	11
	9†	–	6/9	3/9	5/9	6/9	1/9	–	–	+	+	30
	5†	–	ND	ND	3/5	+	–	ND	ND	+	ND	28
MF	6	–	+	+	+	+	–	ND	+	+	+	11
PLTL	1	–	+	–	+	+	+	–	+	+	+	14
	1	ND	+	ND	–	+	–	ND	+	+	+	23
Pag R	1	–	+	+	+	–	–	+	+	+	+	29

Abbreviations:
LCAL, large cell anaplastic lymphoma.
LP, lymphomatoid papulosis.
MF, mycosis fungoides.
PLTL, pleomorphic T-cell lymphoma.
Pag R, pagetoid reticulosis.
TR, transferrin receptor.
B, B-cell markers present.
* Childhood cases.
† Only large atypical cells analyzed.

childhood cases of Ki1 lymphomas[19] parallel the findings in lymph nodes. Primary LCAL mostly affects younger patients with an age peak between the first and second decade.[12,22] In a series of 40 cases[5] skin biopsies were taken from three patients with secondary skin involvement following infiltration of the lymph nodes. These cases were all over 50 years old as were those reported by Ralfkiaer and co-workers[13] and by ourselves. The majority were of secondary LCAL predominantly arising in patients with mycosis fungoides. Thus, LCAL is to be added to the list of high grade malignant lymphomas that may arise at the end stage of MF with the development of skin tumours. Three different clinical types of LCAL of the skin can be distinguished: (i) primary childhood LCAL in the skin; (ii) rare cases of primary LCAL in the skin in older patients; (iii) secondary LCAL in preceding mycoides fungoides or other T-cell lymphomas or secondary after lymph node infiltration.

Ultrastructural studies have been carried out on two cases of cutaneous LCAL.[16,23] Both studies found numerous multivesicular bodies in the cytoplasm which can be regarded as major prelysosomal organelles.[24–26]

CD30 is found on activated proliferating lymphocytes[2,6,27] and is expressed in several cutaneous T-cell lymphomas of high grade malignancy (Table 3) and in nodal lymphomas.[5] Apart from its expression on stimulated normal lymphocytes, a considerable proportion of lymphocytes in angioimmunoblastic lymphadenopathy (AILD), Pagetoid reticulosis and lymphomatoid papulosis (LP) express the CD30 antigen.[5,13,28–30] These disorders are characterized by clonal infiltrates of activated lymphocytes with nuclear and cellular atypia, but normally have a self-limiting, or as in Pagetoid reticulosis a chronic indolent course, or continuously develop new lesions while others disappear as in lymphomatoid papulosis.

We wish to stress that the CD30 antigen is neither exclusively associated with the Hodgkin or Sternberg–Reed cell nor is its expression restricted to malignant lymphomas. We do not agree with the interpretation of Kadin[31] and the subsequent statement of Ralfkiaer and co-workers,[13] that lymphomatoid papulosis, Hodgkin's disease, mycosis fungoides and Ki1/Ber-H2 positive large cell non-Hodgkin's lymphoma all originate from activated lymphoid cells, because the phenotype in a given biopsy does not give information as to the origin of the neoplastic clone, but only to its actual stage of differentiation or activation.

We have presented the histological findings in 10 cases of LCAL arising in the skin, and have shown that LCAL is a definite pathological entity and distinct from other T-cell lymphomas.

ACKNOWLEDGMENT

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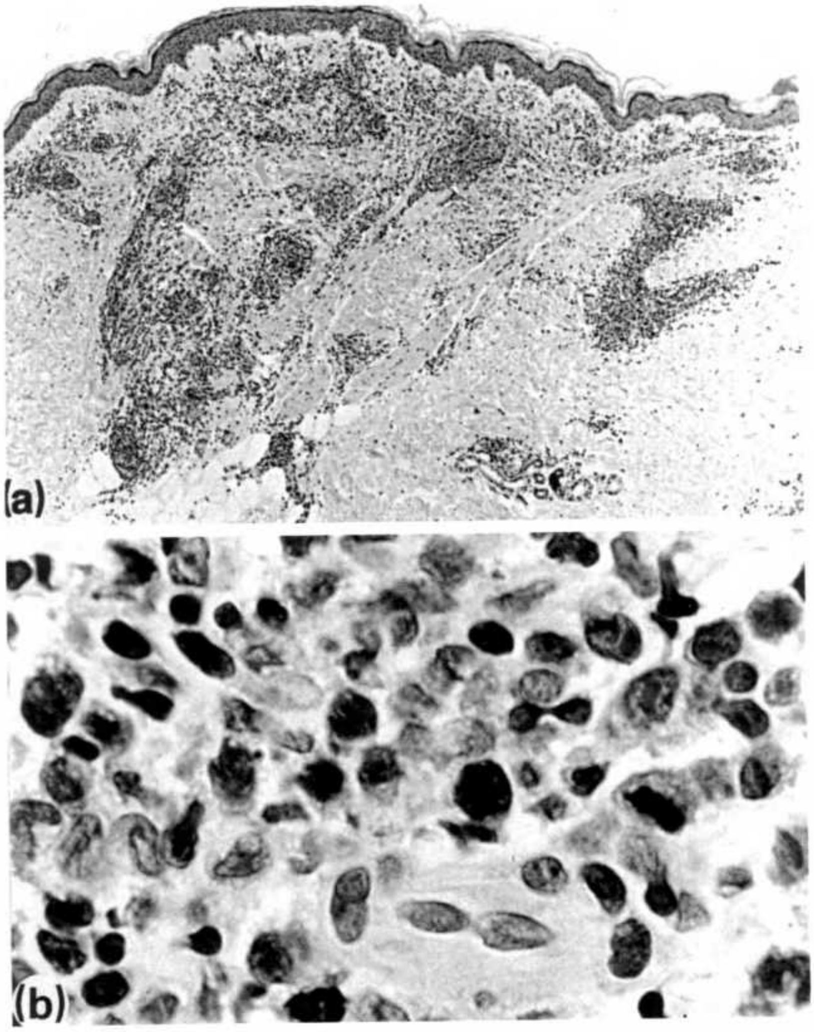

FIGURE 3. Histopathology of case 2, showing neoplastic cells with extreme nuclear pleomorphism and localized around perivascular areas. (a) Low power view (× 75); (b) High power view (× 1000).

Immunohistochemistry

The immunohistochemical findings are summarized in Table 3. In case 1, the majority of neoplastic cells in the tumour nodules expressed both Th/i and T suppressor/cytotoxic (Ts/c) phenotypes with the loss of CD5 antigen. The staining intensity with CD4 was much stronger than with CD8 (Fig. 4).

The percentage of $CD25^+$ cells was higher in the poor prognosis cases, as with $OKT9^+$ cells, apart from case 3. In cases 1 and 3 lymphoblastoid or relatively large neoplastic cells possessed Ki-1 (CD30) antigen. The $CD30^+$ cells lay in a cluster in the tumour nest but were rare in the infiltrate in the perivascular areas. Leu 8 was also detected in the relatively large neoplastic cells in all cases of ATLL (Fig. 5). $CD1^+$ Langerhans cells were usually confined to the epidermis,

TABLE 3. Phenotypes of tumour cells in skin of patients with ATLL

	Percentage of positive cells									
	Case 1									
Antibody	A	B	C	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
CD1	20	3	1	5	15	15	15	25	10	10
CD3	ND	ND	ND	98	85	85	95	ND	90	70
CD4	90	95	95	95	85	80	85	80	70	60
CD5	5	1	1	98	90	85	95	80	60	60
CD8	20	95	95	5	5	30	3	1	50	20
CD11	ND	ND	ND	10	5	5	5	ND	3	15
CD20	2	1	1	8	1	3	5	1	15	3
CD25	95	98	98	95	95	85	90	25	20	10
CD30	1	1	80	15	1	30	1	ND	1	1
CD38	50	5	40	ND	ND	ND	ND	5	ND	ND
OKIa-1	95	90	98	50	90	95	90	95	95	85
OKT9	70	50	70	80	1	65	50	10	55	10
Anti-Leu 8	ND	ND	ND	45	50	55	70	ND	70	15

A, plaque; B, tumour (upper dermis); C, tumour (deep dermis); ND, not done.

RESULTS

Histopathology

Two of our cases were classified as having a medium cell type, two a small cell type, two a pleomorphic cell type, one a large and pleomorphic cell type and one being a mixed cell type. There were two patterns of the infiltration of the skin, being epidermotropic and non-epidermotropic types. Cases 1, 4 and 5 showed epidermotropism of infiltrating cells, and these had Pautrier microabscesses. Atypical lymphocytes in these microabscesses were mostly CD4$^+$ (T helper/inducer) with a few CD1$^+$ cells. In case 1, there was a difference between the cells in the infiltrate of the upper and lower dermis, cells in the upper dermis consisting of small to medium-sized pleomorphic cells with markedly convoluted nuclei, whereas those in the lower dermis were large blastoid cells (Fig. 1). In case 4, there were small lymphoid cells and the perivascular infiltration was associated with evidence of a vasculitis and extravasation of erythrocytes. There were variable sized Pautrier microabscesses in case 5, which showed prominent epidermotropism with a sparse to moderate dermal infiltrate around the blood vessels. Epidermal and transfollicular elimination of lymphoid cells was seen (Fig. 2).

Cases 2, 3, 6 and 7 were of the non-epidermotropic type. In case 2, the infiltrating cells had marked nuclear pleomorphism and were localized around the vessels (Fig. 3). There was a diffuse and massive infiltrate of mononuclear cells with scanty cytoplasm in the upper dermis of case 3 with destruction of the capillaries. The infiltrate contained a few plasma cells, eosinophils and macrophages. In case 6, the infiltrate consisted of small convoluted cells that were around vessels. In case 7, there was a monomorphic cellular infiltrate with pleomorphic nuclei throughout the dermis and extending into the subcutaneous fat. In case 8, all the lymphoid cells had convoluted nuclei and there were histiocytes, eosinophils and plasma cells in the infiltrate.

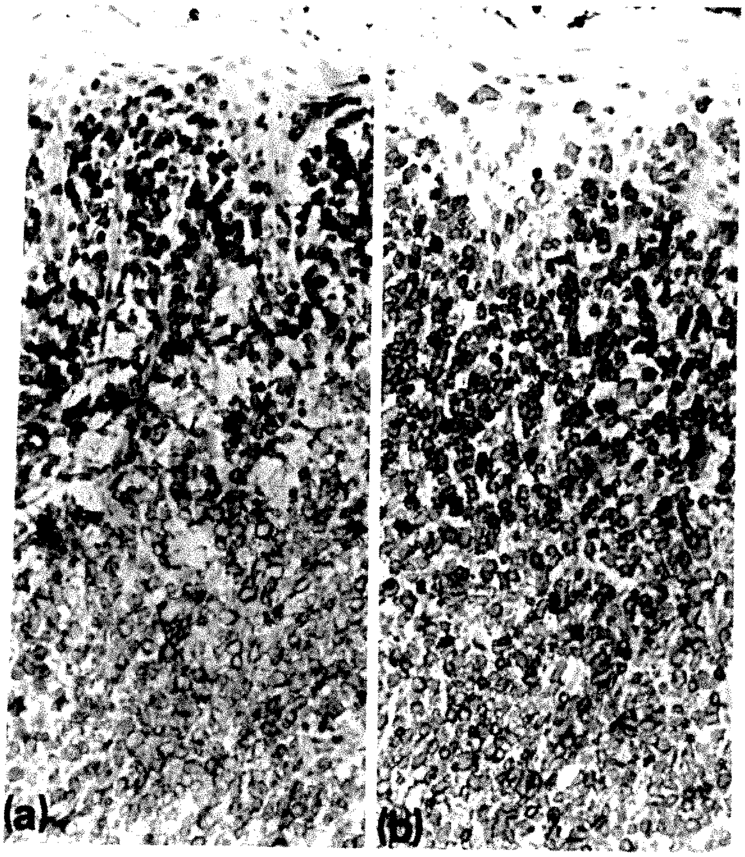

FIGURE 4. Immunohistochemical findings of case 1. (a) CD8 positive cells (× 200); (b) CD4 positive cells (× 200).

but some were found in the dermis in all eight patients. In case 6, about 25% of the infiltrating cells in the dermis were $CD1^+$.

DISCUSSION

In all the cases of ATLL, regardless of their sub-type, antibodies against HTLV-1 and proviral DNA can be detected.[11] In ATLL endemic areas, CTCL may be difficult to distinguish from HTLV-1 positive ATLL with cutanous involvement. In most of our cases the clinical features for ATLL were different from those of MF and SS and most presented with a generalized papular eruption with purpura. Unlike cases of MF, most of our patients had a very short history of several months of skin lesions rather than years. The histological features of ATLL showed several differences from classical MF and SS with marked pleomorphism of the neoplastic cells and few reactive cells such as eosinophils, plasma cells and histiocytes. Also there was damage of the dermal vessels with extravasation of erythrocytes.

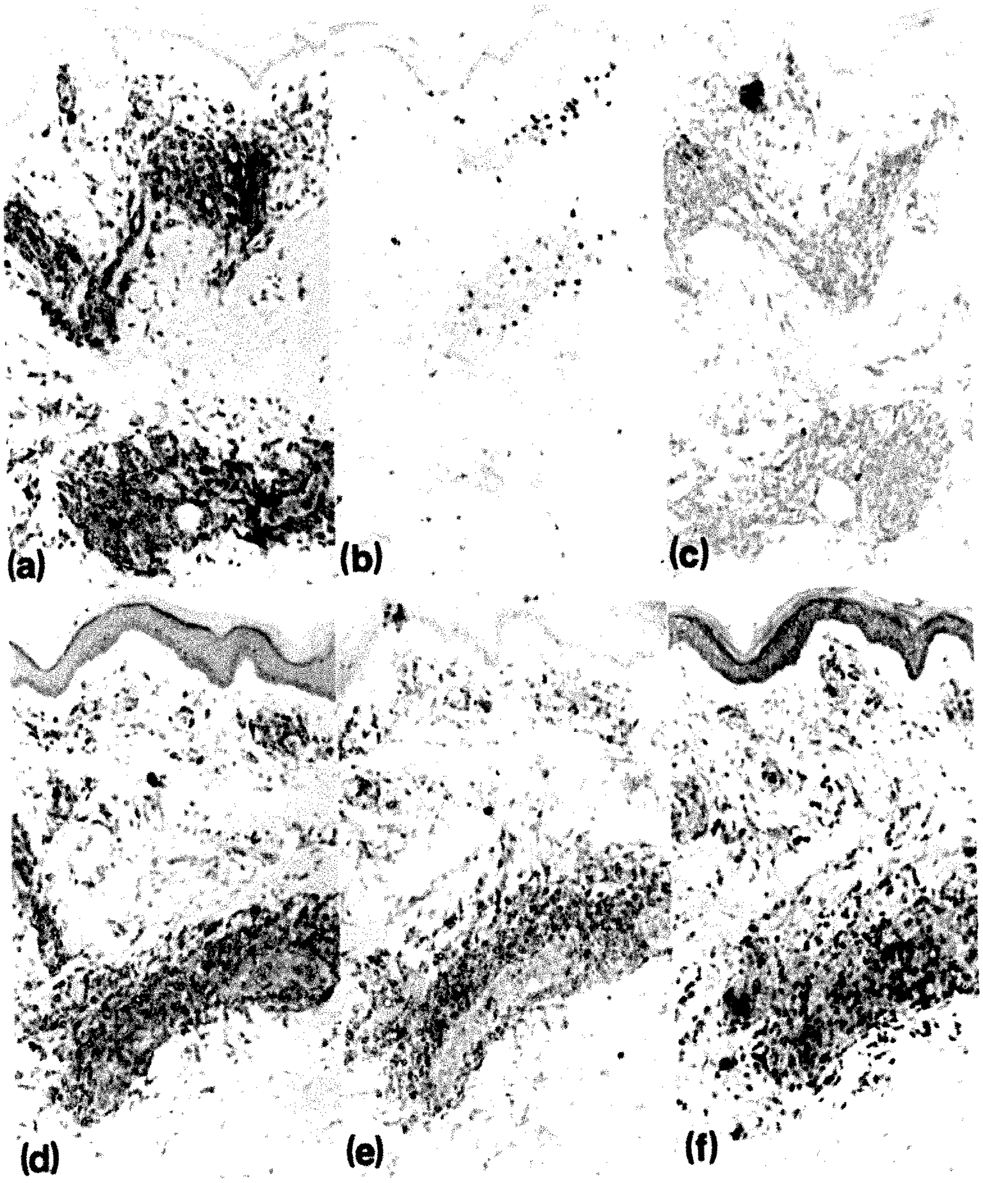

FIGURE 5. Immunohistochemical findings of case 2. (a) CD5, (b) CD8, (c) CD4, (d) CD25, (e) T9, (f) Leu 8 (× 100).

Immunohistochemical studies on skin biopsies from our patients showed a number of common features but also several differences between ATLL and classical MF and SS. CD4/CD8 ratio values were different in the acute, lymphoma (plaque lesion) and the crisis types of ATLL as compared with MF and SS. The erythema or plaque stage of MF usually shows low CD4/CD8 ratios because of the reactive lymphohistiocytic infiltrates, although at the tumour stage of MF there are high CD4/CD8 ratios.[12,13] The mature T-cell antigen phenotypes were almost the same, but there were differences in the expression of activated T-cell markers: Ia, CD25, T9, CD30, CD38 and Leu 8. Higher level expression of CD25 seemed to indicate an acute course and poor prognosis. It has been shown that T cells or T-cell lines that are positive for HTVL-1 often express CD25, whereas other T-cell leukaemia/lymphoma are CD25 negative.[14–16] However, one report found no consistent phenotypic differences between ATLL and CTCL[17] and another found that the expression of CD25 was equally frequent in benign, malignant and HTLV-1$^+$ and negative cutaneous T-cell infiltrates.[18] The activity with CD25 is not confined to T lymphocytes.[19] In our study, 85% and more of the infiltrating cells in the skin in the acute, lymphoma and the crisis types of ATLL reacted with CD25 and less than 5% were CD8$^+$ cells. Although tumour cells from most MF/SS and ATLL cases were the same Th/i phenotype there were quantitative differences in T cell and activating T-cell phenotypes. Despite the similar phenotypes, infiltrating cells in ATLL frequently appeared to function as suppressors of immunoglobulin synthesis, whereas those in CTCL functioned as helper cells.[15] The reactivity of anti-Leu 8 was interesting, and it is known that the major helper effect for antibody formation lies within the smaller CD4$^+$Leu 8$^-$ subset, and that neither CD8$^+$Leu 8$^+$ nor CD8$^+$Leu 8$^-$ cells alone suppress CD4-induced immunoglobulin synthesis.[20–21] A deficiency of Leu 8 antigen has been demonstrated in 18 of 22 specimens from 13 of 15 MF patients.[22] In our study, high levels of Leu 8 antigen were expressed on the relatively large neoplastic lymphoid cells in all the cases of ATLL. This suggests that the neoplastic cells of ATLL may be derived from a CD4$^+$Leu 8$^+$ subset, whereas those of CTCL may be derived from CD4$^+$Leu 8$^-$ subset. A Hodgkin cell associated CD30 antigen has been found on a variable proportion of the cells in lymphomatoid papulosis, angio-immunoblastic lymphadenopathy and certain T-cell lymphomas.[19] In some of our ATLL cases, CD30$^+$ cells lay in clusters in the tumour nest. The vast majority of lymphoblastoid large cells in the lymphoma type of ATLL possessed both CD4$^+$ and CD8$^+$ antigens and expressed CD30 antigen. In addition, CD25$^+$ cells in the cutaneous infiltrates were more numerous than those in the peripheral blood lymphoyctes in the same patient. These observations support the hypothesis that the skin is the major site of extrathymic T-cell differentiation and may promote phenotypic changes in circulating T cells that home in on the skin. There is still a debate as to the role of the skin in the regulation of the proliferation of the infiltrating malignant Th/i cells and the differences seen in ATLL and CTCL may be due to the maturation of the cells in the skin. Other factors play a part, and these include retroviral infection such as HTLV-1, lymphokines, interferons and keratinocyte-derived factors.[23]

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Darier's disease: an immunohistochemical study using antibodies to proteases

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SUMMARY

The expression of plasminogen activators, plasminogen and fibrinogen was investigated in 10 specimens of normal skin and in the involved skin of seven males with Darier's disease, using immunohistochemical techniques. Changes in the localization of proteases were found in the involved skin of Darier's disease, plasminogen being expressed by suprabasal cells and fibrinogen being detected in the keratotic plug.

Mammalian plasminogen activators are highly specific serine proteases which convert the zymogen plasminogen to active plasmin by cleavage of a single peptide bond. A number of cell types have surface receptors for plasminogen and plasminogen activators and some, including fibroblasts and keratinocytes, also secrete plasminogen activators.[1–5] Specific protease inhibitors regulate these activators. Plasmin has a broad substrate specificity and is able to degrade many proteins including extracellular matrix proteins, fibrin and fibrinogen. Two mammalian plasminogen activators have been described: tissue plasminogen activator (tPA) and urinary plasminogen activator or urokinase (uPA). tPA is a fibrin dependent enzyme that plays a major role in vascular thrombolysis, whereas uPA is regarded as a key enzyme in controlling extravascular proteolysis. Plasminogen and fibrinogen are synthesized in the liver and released into the circulation. They appear in the extravascular fluids by exchange processes with the vascular pool. Fibrin, which is formed from its precursor fibrinogen by the action of thrombin, is believed to play a role in many physiological and pathological events and may facilitate keratinocyte migration.[6]

Local secretion of plasminogen activators and activation of plasminogen initiates degradation of extracellular matrix proteins and affects cell migration, cell invasion and tissue remodelling events.[7,8] There is *in vitro* evidence that plasminogen activators are important growth regulators in human epidermis, influencing terminal differentiation, cell detachment and wound healing.[9,10] Activation of plasminogen may also be involved in abnormalities of proliferation and differentiation.[11,12]

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Darier's disease is a dominantly inherited disorder of keratinization characterized by the development of warty papules and plaques in a seborrhoeic distribution. There is evidence of keratinocyte hyperproliferation[13,14] and the histological features include focal areas of acantholysis with formation of clefts and characteristic dyskeratosis resulting in the formation of 'corps ronds' and grains.[15] The primary pathogenetic mechanism is unknown, but ultrastructural studies have demonstrated cytoplasmic vacuolization and breakdown of the complexes between desmosomes and keratin filaments.[16–18]

Plasmin induces acantholysis in skin organ cultures[19] and plasminogen activation has been implicated in the pathogenesis of acantholysis in pemphigus.[20] Proteases may also play a role in the hyperproliferation, abnormal differentiation and acantholysis seen in the skin in Darier's disease. We have therefore investigated the expression of plasminogen activators, plasminogen and fibrinogen in normal skin and the skin in Darier's disease using immunohistochemical techniques.

METHODS

Local ethical committee approval was obtained for this study and informed consent was given by the patients.

Epidermal biopsies

Specimens were examined from seven males with clinical and histologically confirmed Darier's disease. Six patients were using emollients and one patient was taking etretinate 50 mg per day. Punch biopsies of intact keratotic papules were snap frozen and stored in liquid nitrogen until frozen sections were cut. Ten specimens of normal skin collected during routine surgical procedures were also snap frozen and stored.

Antibodies

Polyclonal antibodies were raised in rabbits and characterized in the Oxford Fibrinolysis Research Laboratory.

Two-chain tPA was purified from human melanoma cell culture media by affinity chromatography using immobilized monoclonal antibodies (ESP2, Bioscott, Edinburgh, U.K.). Highly purified high molecular weight uPA was obtained from Hitachi, Japan and purified lysyl plasminogen from Choay Laboratories, Paris. Purified fibrinogen degradation products D and E were prepared in the fibrinolysis laboratory from human fibrinogen (Kabi Vitrum, Uxbridge, U.K.). Fibrinogen degradation products were used instead of fibrinogen since they were purified free of contamination with plasminogen and fibronectin fragments. Each protein was characterized as a single band on analytical polyacrylamide gel electrophoresis performed under non-reducing conditions.

Antibodies were raised in rabbits following repeated injection of sub-milligram quantities of protein in Freunds adjuvent over a period of between 6 and 12 months. The antisera against tPA (W/50), uPA (W/44) and fibrinogen fragments D (W/22) and E (W/23) showed no cross-reactivity with human serum when assessed by double immunodiffusion in agar gels or by immunoelectrophoresis at pH 8·6, confirming the absence of contaminants. Antisera against human plasminogen (W/05), however, exhibited a single precipitin band against human plasma when assessed by the same techniques.

Antibodies against tPA, uPA, plasminogen and fibrinogen fragments were used at dilutions of 1/400, 1/800 and 1/1200 in PBS with 10% normal swine sera (NSS). Unfixed sections were incubated with 30% NSS for 30 min to block non-specific binding. The sections were washed in PBS, incubated for 1 h with the diluted protease antibody, washed and incubated for a further hour with swine anti-rabbit immunoglobulin peroxidase-labelled or fluorescein-labelled conjugate diluted 1/50 in PBS with 10% FCS and 3% human sera. All incubations were carried out at room temperature. After further washes, either colour was developed using diaminobenzidene tetrachloride or the section was examined by fluorescent microscopy.

RESULTS

The results are summarized in Table 1. Similar results were obtained using peroxidase-labelled and fluorescein-labelled conjugates.

Normal skin

Neither tPA nor uPA were detected in normal epidermis, however tPA was present in the basement membrane zone (BMZ) and occasionally was expressed as vertical strands just below the BMZ. Plasminogen and tPA were also detected around dermal vessels. Plasminogen was detected in or on the surface of the basal cells, but was not detected higher in the epidermis (Fig. 1). Fibrinogen was detected diffusely throughout the papillary dermis but was not detected in normal epidermis (Fig. 2).

Darier's disease

There was normal expression of tPA along the BMZ both below acantholytic papules and in adjacent normal skin. tPA was also present around papillary vessels in the dermis. uPA was not detected in the epidermal cells but occasionally there was weak expression in the keratotic plug.

Plasminogen was present not only in the basal cells, but also in many suprabasal cells, acantholytic cells and in the keratotic plug (Fig. 3a, b). There was no suprabasal expression in

TABLE 1. The expression of proteases in normal human skin and skin from patients with Darier's disease

Polyclonal antibody against	Reactivity in normal human skin	Reactivity in Darier's disease
tPA	BMZ and perivascular	BMZ and perivascular
uPA	No staining	Occasional weak expression in keratotic plug
Plasminogen/plasmin	Basal cells	Basal cells suprabasal cells keratotic plug
Fibrinogen fragment (D)	Papillary dermis	Keratotic plug around 'grains' papillary dermis
Fibrinogen fragment (E)	Papillary dermis	Keratotic plug around 'grains' papillary dermis

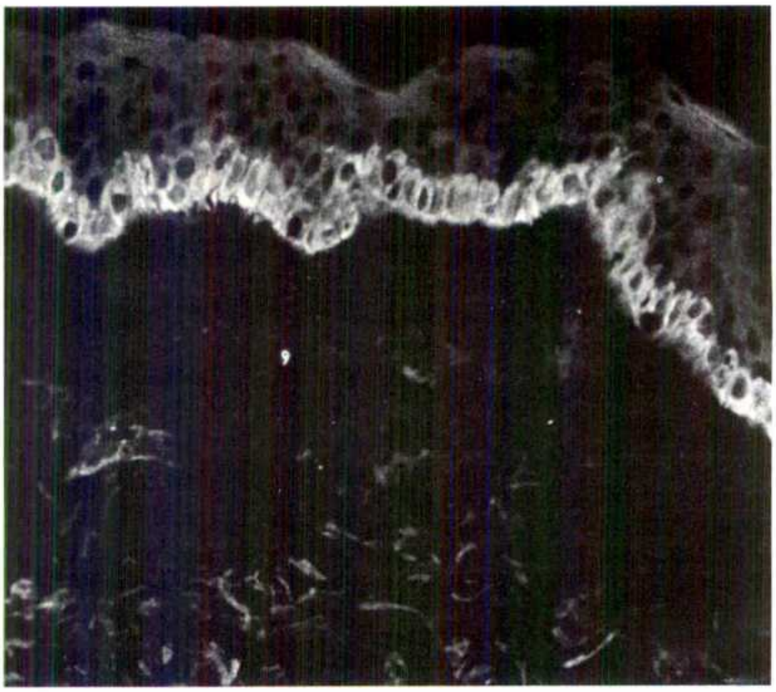

FIGURE 1. Immunofluorescence staining of normal skin with the antibody to plasminogen (W/05). There is intense staining of basal cells but there is no expression on suprabasal cells (Magnification × 340).

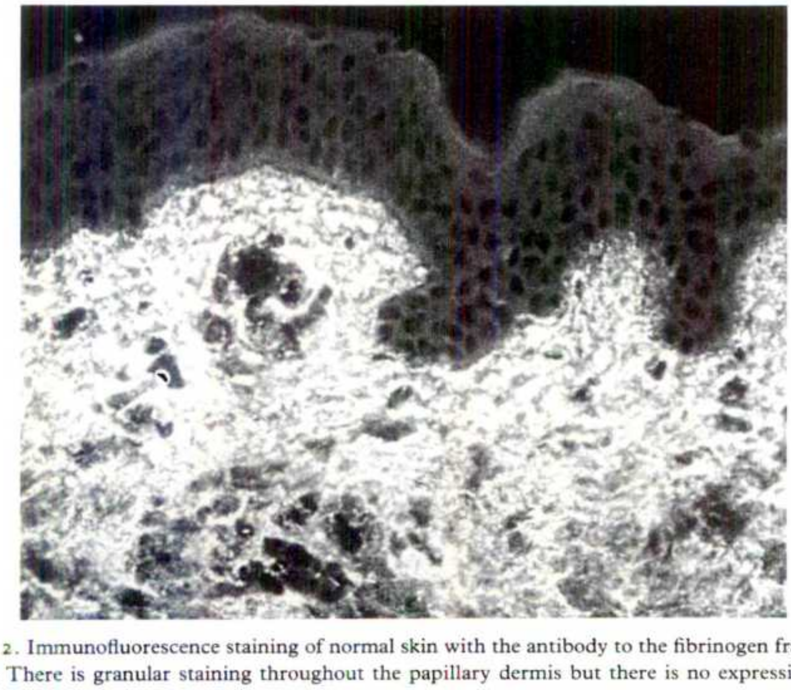

FIGURE 2. Immunofluorescence staining of normal skin with the antibody to the fibrinogen fragment E (W/23). There is granular staining throughout the papillary dermis but there is no expression in the epidermis (Magnification × 340).

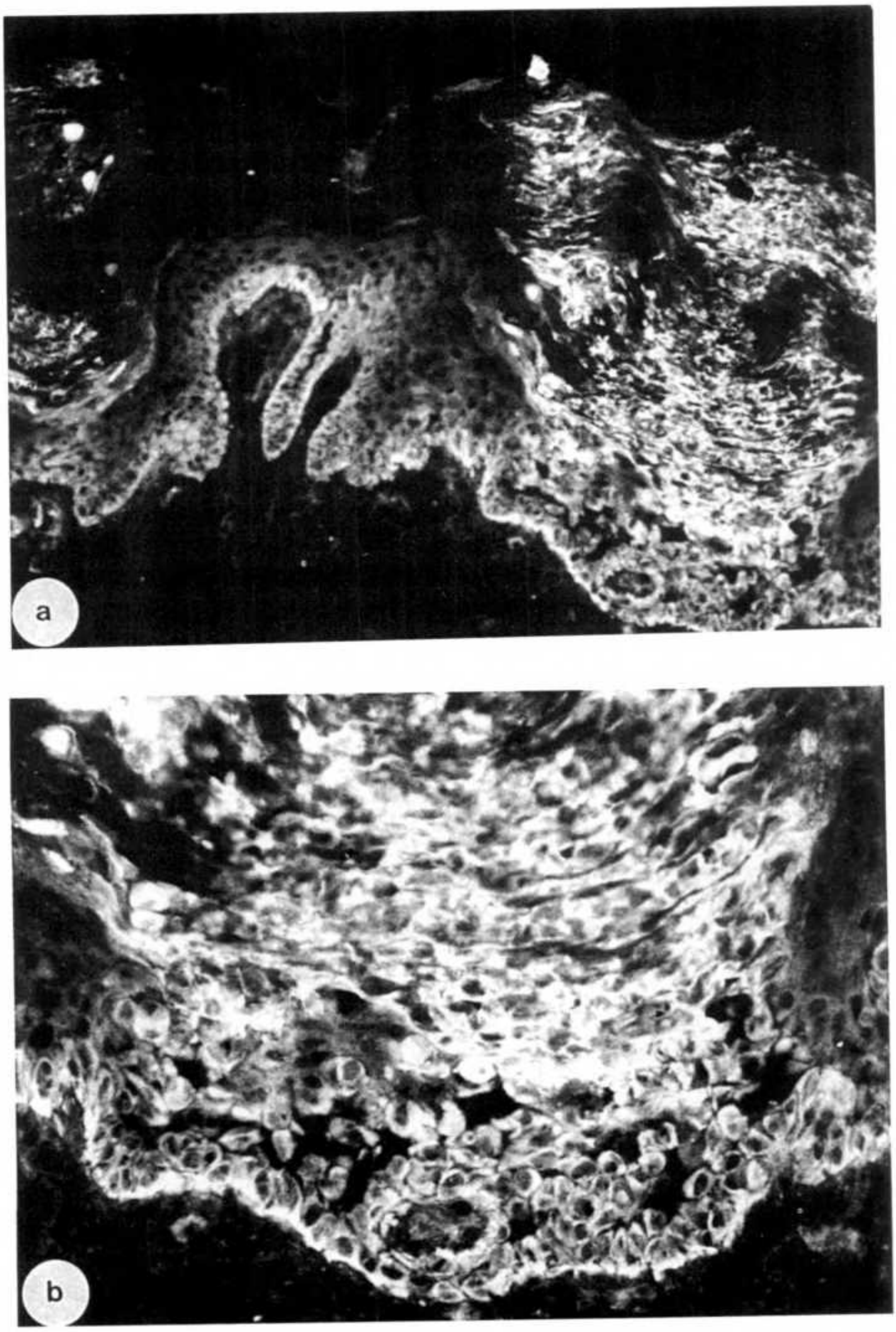

FIGURE 3. (a) Immunofluorescence staining of involved skin in Darier's disease with the antibody to plasminogen (W/05). There is normal staining of the basal cells in uninvolved perilesional skin, but there is intense staining of basal cells, suprabasal cells and the keratotic plug in involved skin (Magnification × 119). (b) Immunofluorescence staining of involved skin in Darier's disease with the antibody to plasminogen (W/05). The basal and suprabasal acantholytic cells in the Darier's lesion fluoresce strongly (Magnification × 280).

perilesional non-involved skin. In one patient with severe erythrodermic Darier's disease, plasminogen was present mainly in the high suprabasal cells. This patient was taking etretinate.

Fibrinogen was present high up in the Darier's lesion throughout the keratotic plug outlining the 'grains' (Fig. 4). There was no expression in the adjacent non-involved epidermis. Fibrinogen was also detected throughout the papillary dermis as it was in normal skin.

DISCUSSION

The expression of plasminogen in normal epidermis was restricted to cells of the basal layer. This observation is consistent with previous reports.[21] The absence of reactivity in suprabasal cells suggests that, during differentiation, binding is masked by conformational changes, or that plasminogen is consumed or inhibited during maturation and upward migration of basal cells. However, in the involved skin in Darier's disease, plasminogen was detected not only in the basal layer but also in many suprabasal cells, acantholytic cells and the keratotic plug. Plasminogen has also been found in suprabasal cells and the parakeratotic stratum corneum in

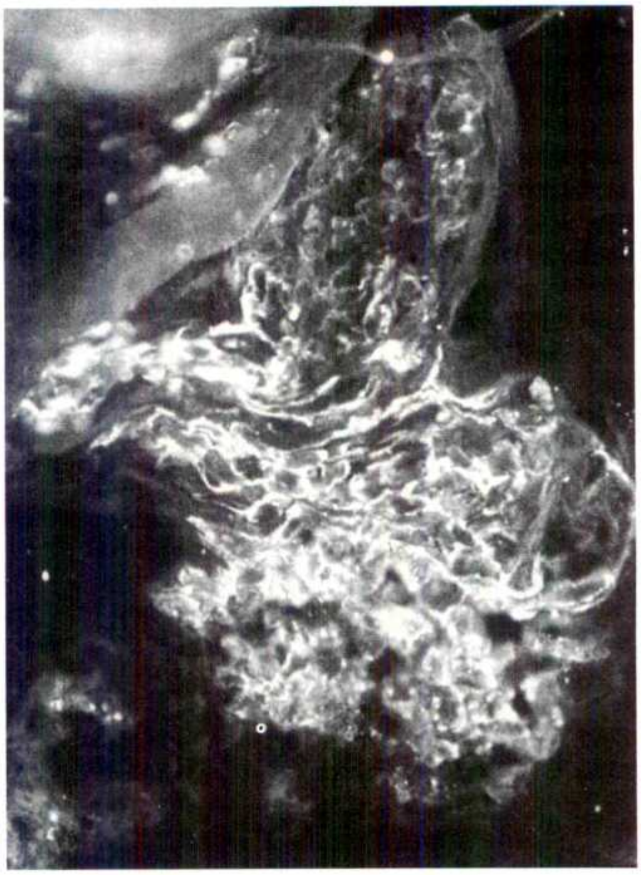

FIGURE 4. Immunofluorescence staining of involved skin in Darier's disease with the antibody to fibrinogen fragment D (W/22). There is staining in the papillary dermis and bright staining outlining the 'grains' in the keratotic plug (Magnification × 340).

the skin in psoriasis[22,23] and there is an increase in activity of plasminogen activator in the skin in psoriasis.[11] Activation of the plasminogen system may stimulate cell turnover and migration. Rapid keratinocyte proliferation is a feature of psoriasis, and keratinocyte hyperproliferation is also found in Darier's disease.[13,14]

Many of the suprabasal cells which expressed plasminogen were also acantholytic. Plasmin induces acantholysis in skin organ cultures[19] and in pemphigus, and activation of the PA-plasminogen system has been implicated in the pathogenesis of acantholysis.[20] There is recent evidence that surface binding of uPA colocalises with vinculin, an intracellular actin-binding protein that is deposited at cell-to-substratum focal adhesion sites.[24] The association between a plasminogen activator, sites of cell adhesion and cytoskeletal attachments provides further evidence for a link between plasmin release and the regulation of cell-cell adhesion, cell morphology and cell movement. Some suprabasal acantholytic cells in the skin in Darier's disease express keratin markers normally restricted to basal cells.[14] This may be secondary to abnormal aggregation or degeneration of keratin filaments. If plasminogen was activated at sites of cytoskeletal attachment in these cells, this could not only break down keratin filament–desmosomal complexes and cause acantholysis but might also induce secondary changes in cytoskeletal keratin conformation with changes in epitope expression. Thus, the presence of increased amounts of plasminogen in the skin in Darier's disease may play some part in both the acantholytic process and the dyskeratosis.

Increased plasminogen conversion may be secondary to increased release of PA or to decreased production or defective function of PA inhibitors, but the antibody used in this study cannot distinguish active plasmin from its inactive precursor and it is not possible to decide whether these cells have higher surface proteolytic activity or only an increased potential for proteolysis.

The antisera against fibrinogen fragments (W/22 and W/23) react with epitopes on intact fibrinogen as well as degraded fibrin, and although it is not possible to determine whether the fibrinogen was plasmin degraded, binding probably indicates the presence of intact fibrinogen, rather than discrete degradation products.

Fibrinogen was not detected in normal epidermis, but it was present throughout the papillary dermis. This supports the view that fibrinogen diffuses from the dermal capillaries into the surrounding connective tissue. However, in the skin in Darier's disease, fibrinogen was also detected in the keratotic plug. In preliminary observations we reported that fibrinogen was present in the parakeratotic stratum corneum in psoriasis.[23] It is not clear why fibrinogen is detected in areas of abnormal keratinization, but fibrinogen and fibrin are important in a wide range of physiological and pathological processes. The role of fibrin in haemostasis is well known, but fibrin is also believed to play some role in healing wounds and invading tumours, where it may facilitate cell movement.[6] Fibrinogen is present in chronic inflammation, when fibrinogen as well as fibrin and their fragments may induce retraction of vascular endothelial cells, increase vascular permeability and attract leucocytes.[6] The presence of fibrinogen in the parakeratotic horny plugs in involved skin in Darier's disease may reflect decreased levels of PA and thus, a delay in both fibrinolysis and protease-mediated nuclear degradation. Alternatively, focal fibrinogen deposition may be secondary to local release of inflammatory mediators.

The distribution of plasminogen activators in the skin in Darier's disease is essentially normal except for trace amounts of uPA occasionally associated with the keratotic plug. In a previous study we reported that uPA was present in the parakeratotic horny layer in psoriasis.[23] There is *in vitro* and *in vivo* evidence that uPA has a role in keratinocyte differentiation and desquamation.[4,9,10]

We conclude that the skin is capable of generating proteolytic activity in discrete regions and we have confirmed previous studies showing that plasminogen is present in the basal layer of the normal epidermis. Our study shows that there are changes in the immunohistochemical localization of proteases in the skin in Darier's disease, in particular plasminogen is expressed by suprabasal cells and fibrinogen is detected in the keratotic plug. The increased expression of plasminogen is considered to be a consequence of an increase in plasminogen receptors rather than local synthesis. These changes may be secondary to the abnormalities in cell differentiation and proliferation in Darier's disease, but alterations in the balance between protease activators and inhibitors or changes in the localization or availability of proteases may play a role in the pathogenesis of hyperproliferation, dyskeratosis and acantholysis in the skin in Darier's disease.

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Strategy of acne therapy with long-term antibiotics

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SUMMARY

A study of the outcome of conventional antibiotic treatment in 543 patients with acne was performed. All were treated initially with erythromycin 1 g/day and topical 5% benzoyl peroxide. The median improvement at 6 months was 78%, with an interquartile range of 67–90%. Four-hundred and eight of the 492 who completed 6 months' treatment showed over 50% improvement and 247 of the 279 patients treated for a subsequent 6 months with benzoyl peroxide alone, continued to do well. Another subgroup of 174 patients, was continued for 6 months with systemic antibiotic and 5% benzoyl peroxide. No significant benefit was gained, however, by maintaining successfully treated patients on a further 6 months of systemic antibiotics. Of the 84 patients who did less well, 18 were given alternative treatment (Diane®, isotretinoin). The other 60, subsequently referred to as slow responders, were continued on antibiotics (erythromycin, 31; minocin, 29; cotrimoxazole, 4; trimethoprim, 2) and benzoyl peroxide. Those prescribed minocycline for the second 6 months appeared to have greater benefit (64%) than those receiving erythromycin (57%). This level of improvement was still lower than that seen in those who responded well within 6 months (78%). Of the risk factors analysed, the poorest response occurred in males with truncal acne. Age at presentation, duration and severity did not adversely affect therapeutic outcome. Side-effects were minimal.

One of the mainstays of acne therapy continues to be systemic antibiotics. Despite their use for many years, treatment regimens with antibiotics remain empirical. Greenwood *et al.*[1] showed that patients on 1 g/day of erythromycin did better than those on 500 mg/day and that their relapse rate was reduced, and on the lower dose regime, it was found that certain subgroups of patients (severe acne, truncal acne and male patients) had less satisfactory results. This study investigated whether the same oral treatment, continued for longer than 6 months, produced even further benefit; whether the higher dose of antibiotic eliminated the tendency of some patients to do less well; and if patients who did not respond well showed a significant improvement when given an alternative antibiotic regime.

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METHODS

All patients attending the out-patient clinic for treatment of their acne who were suitable for antibiotic therapy were studied. This excluded physiological acne or severe nodulocystic acne. Patients were asked to stop all therapy for the month prior to attending for the first out-patient visit. The patients were treated with an initial 6 months of erythromycin 1 g/day and topical 5% benzoyl peroxide (Panoxyl Aquagel No. 5).

Following the initial 6 months of therapy, the patients were treated in one of three ways, according to the response of the acne to treatment: (i) Five per cent benzoyl peroxide alone for 6 months; (ii) erythromycin 1 g/day and 5% benzoyl peroxide for a further 6 months; or (iii) an alternative antibiotic and 5% benzoyl peroxide for a further 6 months. Patients who responded well were continued on the same medication for a further 6 months or the antibiotic was stopped and the patient maintained on benzoyl peroxide alone. Those patients who did not do well were treated for the second 6 months with the same or an alternative oral antibiotic. Instructions were given to each patient regarding therapy with erythromycin being taken half-an-hour before meals and minocycline with meals. Benzoyl peroxide gel was applied to all areas daily and the frequency reduced if redness or peeling occurred.

The patients attended clinic at 3-monthly intervals, but the data was only analysed at the 6 and 12 month visits. The patients were seen by the same doctor at each visit and their acne graded.[2] All doctors who participated in the study had obtained instruction in grading and correlation was ensured prior to starting the study. Patients were questioned about compliance and side-effects from the medication. Patients were withdrawn if not complying, or if the records of their acne grades were not complete.

Analysis of patients who had two antibiotics over a 12-month period was restricted to those who had been treated with erythromycin for 6 months followed by minocycline (200 mg/day) for 6 months. The numbers of patients who were treated with other antibiotic regimes (cotrimoxazole, tetracycline and trimethoprim) were too small for assessment and so were not included.

Non-parametric statistics were used. Effect of treatment was assessed by percentage improvement of the acne grades at 6 and 12 months, compared to the pre-treatment values. Failure of treatment was defined as an improvement of less than 50%, or a total acne grade after treatment of greater than 3 after 6 months' treatment. Relapse was defined in the successfully treated patients as a return at 12 months of the acne severity to greater than 50% of the original acne grade.

When analysing the data for the risk factors, i.e. age at presentation, duration of symptoms, severity of acne, sex of patient, and site of acne, the patients were matched for all factors other than the one being assessed. The patients were defined as having truncal or facial acne, according to the most severe site. If both were of equal severity, the patient was not included in the analysis.

Significance of incidence of side-effects between the two groups was analysed using the χ^2-test.

RESULTS

Five-hundred and forty-three patients were commenced on erythromycin and benzoyl peroxide and 492 (276 male, 216 female) completed 6 months' treatment. Fifty-one patients either failed to attend for follow-up or were excluded for non-compliance. The median values and

TABLE 1. Efficacy of treatment (data presented as median with interquartile ranges in parentheses)

Number of patients	% improvement at 6 months	Second line therapy	Number of patients	% improvement	% relapse*
492	77·8 (67–90)	BP alone	279	77 (63–88)	12
		E+BP	139	74 (57–88)	15
		M+BP	32	65 (41–82)	—

* Excluded 'failures' at 6 months.
BP, benzoyl peroxide.
E, erythromycin.
M, minocycline.

interquartile range for the whole group for age at presentation was 18 years (15–25 years); duration of acne was 4 years (2–7 years); and the severity mean grade was 2 (1·5–3·5). Three-hundred and thirty patients had predominantly facial acne and 124 had predominantly truncal acne.

The median percentage improvement at 6 months was 77·8, with an interquartile range of 67–90%. Of the 492 patients, 84 failed to achieve a 50% improvement in their acne. One-

TABLE 2. Analysis of risk factors for a poor prognosis expressed as percentage improvement

Risk factor	Number of patients	Median % improvement	Significance*
Age (years)			
$\leqslant 18$	72	80	$P=0{\cdot}9$
>18	72	78	
Duration (years)			
$\leqslant 4$	104	79	$P=0{\cdot}7$
>4	104	80	
Severity			
$\leqslant 2$	133	76	$P<0{\cdot}02$
>2	133	81	
Sex			
Male	134	78	$P<0{\cdot}03$
Female	134	82	
Predominant site			
Facial	102	87	$P<0{\cdot}005$
Truncal	102	76	

* Mann–Whitney *U*-test.

TABLE 3. Results in patients treated with antibiotics for 12 months (interquartile range in parentheses)

Treatment for second 6 months	Number of patients	Median % improvement at 6 months	Median % improvement at 12 months	Significance
Minocycline +BP	29	28 (16–44)	64 (40–83)	$P\leqslant 0{\cdot}002$*
Erythromycin +BP	31	37 (20–46)	57 (32–64)	$P\leqslant 0{\cdot}02$*

* Wilcoxon signed rank test.

hundred and thirty-nine were continued on the same treatment, 279 were just given benzoyl peroxide alone and 32 were given minocycline and benzoyl peroxide. The median percentage improvement and interquartile ranges and relapse rates at 12 months of these three subgroups are shown in Table 1. No significant change in acne severity occurred at 12 months, compared to 6 months, in those patients maintained on benzoyl peroxide alone for the latter 6 months, or treated in addition with erythromycin (Wilcoxon signed rank test). Those patients who were changed to minocycline tended to be 'failures' and so it was not appropriate to compare their outcome at 12 months.

The data for analysis of risk factors for a poor prognosis after 6 months of therapy are shown in Table 2. Age at presentation and duration of acne were not significant factors in assessing outcome. Those patients with more severe acne improved more than those with lower acne grades ($P\leqslant 0{\cdot}02$). Male patients compared to female patients ($P\leqslant 0{\cdot}03$), and patients with predominantly truncal acne rather than facial acne ($P\leqslant 0{\cdot}005$), did significantly less well.

Of the 84 patients who did not achieve greater than 50% improvement, 66 were continued on oral antibiotics (erythromycin, 31; minocycline, 29; cotrimoxazole, 4; trimethoprim, 2). The other 18 were given either Diane® or isotretinoin. The outcome of those patients treated subsequently with erythromycin or minocycline is shown in Table 3. Both groups were

TABLE 4. The percentage of patients experiencing side-effects while on antibiotics (numbers of patients in parentheses)

Side-effect	Erythromycin (492)	Minocycline (32)
Diarrhoea	7 (34)	3 (1)
Colic	7 (32)	3 (1)
Nausea	2 (9)	6 (2)
Indigestion	<1 (3)	0 (0)
Vaginal thrush	2 (10)	3 (1)
Headaches	<1 (1)	6 (2)
Dizziness	<1 (1)	6 (2)
Pigmentation	0 (0)	6 (2)

significantly improved after the second 6 months of treatment. The percentage improvement was greater in those changed to minocycline. As part of our clinical assessments, side-effects are routinely detailed. The side-effects were minimal and are shown in Table 4. Minocycline produced significantly more neurological symptoms (headache/dizziness) than erythromycin ($P \leqslant 0{\cdot}01$). Pigmentation of acne scars and/or on the lower leg, was only seen with minocycline.

DISCUSSION

Our study showed that if a patient responded well after 6 months of oral and topical therapy, there was no further benefit from continuing antibiotic therapy. The patient's level of acne could be reasonably maintained on topical therapy (benzoyl peroxide) alone. Secondly, we have shown that a high dose of antibiotics (1 g erythromycin and 5% benzoyl peroxide) eliminates certain risk factors (age, duration and severity of acne), the latter of which adversely influences the therapeutic response with lower doses (0·5 g erythromycin and 5% benzoyl peroxide). However, in some patients (16%) who slowly respond to treatment, continuation of either the same (erythromycin) or alternative antibiotic (minocycline) produces, after a further 6 months' therapy, a significant improvement. The data suggests that an alternative antibiotic may have a better effect. This needs to be addressed further in a prospective clinical trial.

There are many clinical trials showing the effects of various acne treatments[3–5] but few studies performed in the routine dermatological clinic and which assess the in-use benefit and problems of conventional acne therapy. In a previous study,[1] we showed that patients given erythromycin 1 g/day and benzoyl peroxide for 6 months showed a relapse rate of 35% 12 months after stopping therapy. In this present study our relapse data was 12% at 6 months, which excluded the failures after 6 months' treatment. In both studies, independent of the dose, patients with predominantly truncal acne responded less well than those patients whose acne was mainly confined to the face. The two doses 0·5 and 1 g had a different effect when analysed with respect to acne severity. On the lower dose, patients with less severe acne did best, but on the higher dose a greater percentage improvement was seen in those with more severe acne. Thus, if a patient requires oral antibiotics, then 1 g of erythromycin and benzoyl peroxide should be given rather than the 0·5 g dosage and benzoyl peroxide. Side-effects in our patients were as expected and were minimal.

We studied erythromycin rather than tetracycline as we wished to compare our data with previous studies, and because it is better absorbed. It is possibly a safer drug than tetracycline, should the patient be on the pill or become pregnant. We have shown that 0·5 g doses of tetracycline and erythromycin are equally effective and that the addition of 5% benzoyl peroxide does have a significant additional effect.[6]

The protocol of this study was designed prospectively, but allocation of patients to the groups for treatment was not random which prevented between-group analyses. Acne grading is a subjective assessment, but the numbers of subjects in this study and the need to look at the severity of the truncal as well as facial acne precluded the use of lesion counts, which is much more time consuming but more objective. The same doctor assessed each patient at sequential visits, which permitted maximum consistency with grading.

Some patients failed to respond to antibiotic therapy and there were more males in this group than females and more with truncal acne. It could be argued that topical treatment was not applied to the trunk, but when patients are treated with isotretinoin their truncal acne responds less well.[7] The rate of sebum excretion may be important, and two clinical studies noted a poorer response to antibiotic therapy in those patients with a high sebum excretion rate (SER).[1,8] It was suggested that a high SER could cause dilution of the antibiotic, to a suboptimal level.[8] There may be some virtue in giving such patients even larger doses of antibiotics.

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Symptomatic dermographism: wealing, mast cells and histamine are decreased in the skin following long-term application of a potent topical corticosteroid

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SUMMARY

Clobetasol propionate 0·05% ointment and an otherwise identical steroid-free base were applied topically to a 10 cm^2 area on the anterior thighs of six patients with symptomatic dermographism for 6 weeks. Four patients showed a significantly decreased wealing response to stroking of steroid pretreated skin compared to that of control sites. There was a parallel decrease in mast cell numbers and histamine levels in skin biopsies taken from the steroid treated areas. At 6 weeks two patients demonstrated a decrease in flare areas following the intradermal injection of compound 48/80 in steroid pretreated skin compared to base treated sites. Flare areas following intradermal injection of histamine in these two patients were equivalent in base and steroid treated skin.

The itching and wealing of the physical urticaria, symptomatic dermographism (SD), occur in response to stroking of the skin. Much of the itching, erythema and oedema is due to histamine released from mast cells, which have been shown to degranulate and there is an associated increase in plasma histamine levels.[1]

In normal skin long-term application of topical corticosteroids produces a potency-related decrease in skin histamine levels associated with a reduction in the number of mast cells.[2] Application of a potent topical steroid to normal skin 14, 4 and 2 h before testing suppressed the erythematous flare produced by the mast cell degranulating agent, compound 48/80, given intradermally, in a study in which the wealing response was not documented.[3] In urticaria pigmentosa the increased numbers of identifiable mast cells are similarly reduced following

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corticosteroid applications.[4] We investigated the effect of topical clobetasol propionate 0·05% on wealing, mast cell numbers and skin histamine levels in SD, a form of physical urticaria where a wealing tendency is not associated with an increased number of mast cells.[1,5]

METHODS

Patients

The diagnosis of SD was based on a history of an itching and wealing response to stroking, and a similar response to stroking with a dermographometer[6] calibrated at pressures of $2{\cdot}0 \times 10^5$ (20·4 g/mm^2), $5{\cdot}87 \times 10^5$ (59·9 g/mm^2), $9{\cdot}75 \times 10^5$ (99·4 g/mm^2) and $14{\cdot}12 \times 10^5$ (144 g/mm^2) pascals. An itchy wealing response to a pressure of $3{\cdot}5 \times 10^5$ Pa (35·7 g/mm^2) or less,[7,8] necessary to confirm the diagnosis of SD, was present in all patients.

Six volunteer patients with SD (5 males) attending St John's Hospital for Diseases of the Skin, completed this study for which ethical approval was obtained. All gave informed written consent. Ages ranged from 24 to 58 years (mean 43 years) and their SD had been present between 1·5 and 14 years (mean 5·25 years). None suffered from any other form of urticaria or other intercurrent disease. Antihistamine therapy was stopped 72 h prior to each assessment. None were taking long acting antihistamines or any other medication.

Clinical assessment and methods

All six patients were seen on three occasions: the initial visit, at 3 weeks and at 6 weeks. Two patients returned 32 weeks after the initial visit for further assessment. During the initial visit clinical assessment was performed by holding the dermographometer perpendicularly to the upper back and stroking longitudinally at pressures of $2{\cdot}0 \times 10^5$, $5{\cdot}87 \times 10^5$, $9{\cdot}75 \times 10^5$ and $14{\cdot}12 \times 10^5$ Pa. As all six patients responded to the lowest pressure by itching and wealing 5 min later, they were included in the study.

Two 10 cm^2 areas on each anterior thigh were defined by shaving. The dermographometer was then used to stroke the skin transversely within both defined areas at each pressure. Five minutes after stroking, the width of each resulting linear weal was measured in mm at three points along the length of the weal and the mean of three measurements taken for each pressure. The surrounding flare was not measured. Clobetasol propionate 0·05% (CP) and an otherwise identical ointment base from which the steroid had been omitted were obtained from Glaxo U.K. Ltd, Greenford, Middlesex, U.K. Patients were instructed to apply one ointment to the right and the other to the left 10 cm^2-designated area on each thigh twice daily for 6 weeks. The applications were randomized[9] and the study was carried out double-blind. Both areas were occluded at night using sheets of polythene kept in place with micropore tape and Tubifast (Seton, Oldham, U.K.). Patients were requested to regularly shave the hair in the test areas.

At 3 weeks all 6 patients were reviewed. The test areas were examined for any evidence of atrophy (telangiectasia visible on stretching of the skin or any loss of skin substance indicating dermal atrophy.) Dermographometer assessment of wealing was repeated as previously. Since the weal width at 3 weeks was little different from that at the initial visit these values were not included in the results. Patients were given further supplies of both ointments, micropore tape and Tubifast.

At 6 weeks, a 4 mm punch biopsy was performed in four of the patients using 2% lignocaine, without adrenaline, as local anaesthetic in both steroid and base treated areas. After both skin biopsies had been performed, dermographometer assessment of wealing was performed in the treated areas. The change in weal width after 6 weeks treatment with clobetasol propionate

0·05% compared to the control area was analysed using the paired *t*-test. Two of the four patients who had volunteered to have skin biopsies performed returned for two further skin biopsies at 32 weeks; the two other patients declined. The remaining two of the original six patients were given intradermal injections of compound 48/80 (Sigma Ltd, Poole, Dorset, U.K.) 0·1 μg, 1·0 and 10 μg in 0·05 ml/0·15 M sodium chloride into the treated areas. Histamine 0·1 μg, 0·05 ml and 0·15 M sodium chloride was also given intradermally. The resulting flares were traced onto the plastic and the flare area was later measured in cm^2 using a planimeter (Allbrit, W.F. Stanley, London U.K.).

Mast cell counts

Each skin biopsy was bisected, half being kept for histamine estimation and the other half placed in formal saline for mast cell counting. The mast cells were identified using the chloroacetate esterase reaction.[10] Two independent observers counted all mast cells from 10 high power fields (HPF), six HPF of epidermis and upper dermis and four HPF of mid and deep dermis, at a magnification of 400 × (Zeiss microscope) in the sections from base- and steroid-treated areas. The mean count of both observers over 10 HPF was obtained and statistical analysis performed using the paired *t*-test.

Histamine extraction and estimation

Skin histamine content was measured in both steroid- and base-treated samples using a single isotope radioenzyme assay.[11] The tissue was freeze dried for 24 h in dried capped preweighed polypropylene tubes and stored at −20°C prior to histamine extraction. To extract the histamine, 200 μl 3% perchloric acid was added to each tissue specimen and boiled for 10 min in a water bath. Phosphate buffer pH 7·9 (800 μl 0·2 M) was then added, vortex mixed, sonicated for 10 min and then centrifuged at 2000 ***g*** for 8 min using an Eppendorf centrifuge. The supernatant was aspirated and stored at −20°C. Histamine was converted to methyl histamine using histamine-*N*-methyl transferase extracted from rat kidney in the presence of [^{3}H]-labelled S-adenosyl methionine (Amersham).[11] The assay was modified by taking 50 μl aliquots of each sample and adding 5 μl of diamine oxidase 1 IU/ml in assay buffer to each one. These aliquots were assayed in parallel with the non-digested samples to correct for variation in non-specific background methylation. The limit of detection of histamine was 0·05 ng/ml. The resulting histamine levels in ng/ml free base were expressed as ng/mg per dry tissue weight. Statistical analysis was performed using the paired *t*-test.

RESULTS

Clinical findings

The wealing reaction on stroking the skin was diminished by the application of the topical steroid (Fig. 1). Wealing was absent at the lowest pressure and the weal width was greatly reduced at higher pressures. Weal width and pressure were more directly related when the two lower pressures were applied. Mean weal widths in mms were 4·05 ± 0·20, 5·41 ± 0·56, 5·08 ± 0·47, 5·33 ± 0·65 and 0·0, 0·41 ± 0·41, 0·25 ± 0·25, 1·41 ± 0·90 pre- and post-clobetasol propionate respectively at pressures of 2·0; 5·87; 9·75 and $14·12 \times 10^5$ Pa/mm^2 (mm; $n = 4$; mean ± SE). Mean weal widths pre- and post-base application were 3·99 ± 0·49, 4·66 ± 0·40, 5·16 ± 0·67, 5·33 ± 0·89; and 3·83 ± 0·28, 3·58 ± 0·20, 3·66 ± 0·43, 4·33 ± 0·48 respectively (mm; $n = 4$; mean ± SE) for the same pressures. The reduced mean weal width after the application of base may be related to a decrease in shearing force secondary to the frequent use of the emollient

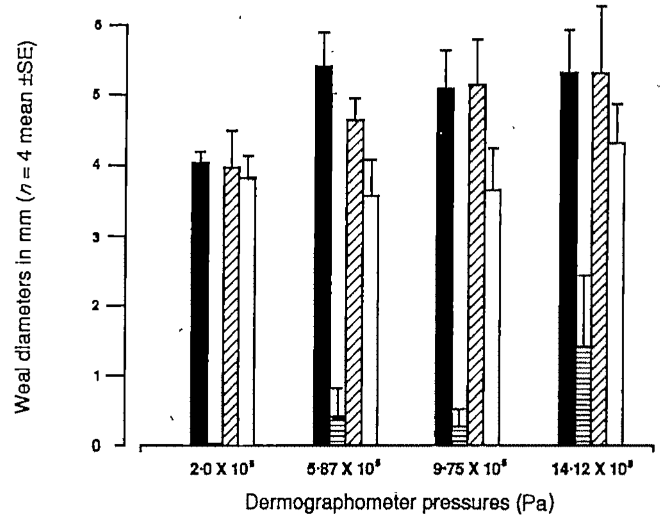

FIGURE 1. Wealing pre- and post-application of clobetasol propionate 0·05% and ointment base. ■, pre-clobetasol; ▤, post-clobetasol; ■, pre-base; □, post-base.

base. At each pressure there was a significant difference ($P < 0{\cdot}05$, paired *t*-test) between the changes in weal width after clobetasol treatment and those after base treatment. Flare width was not measured. Itching occurred in the wealed, stroked areas but was not a prominent symptom after the wealing response had been reduced by application of the steroid.

Mast cell numbers and skin histamine levels

Mast cell numbers in skin biopsies were markedly decreased after 6 weeks pretreatment with CP though not entirely absent. Mean mast cell numbers were 19·5 ± 3·84 and 1·75 ± 1·18 for base-treated (control) and steroid-treated sites respectively. ($n = 4$; 10 HPF; mean ± SE) (Table 1). The decrease was significant ($P < 0{\cdot}02$; paired *t*-test). In the two patients who returned for repeat skin biopsies at 32 weeks, mean mast cell numbers per 10 HPF in each biopsy were similar (18/10 HPF in previously steroid-treated skin and 21/10 HPF in previously base-treated skin).

Skin histamine levels decreased in parallel with mast cell numbers after 6 weeks application of CP compared to levels after 6 weeks application of base although these differences were less striking. Mean skin histamine levels were 1·10 ± 0·47 (ng/mg per dry weight skin, ± SE) post-steroid application and 4·50 ± 1·70 (ng/mg per dry weight skin, ± SE) post-base (control) application. The change was significant at the level of $P < 0{\cdot}05$. Mean histamine levels in skin biopsies at 32 weeks were 6·95 ng/mg dry weight in steroid pre-treated and 14·55 ng/mg dry weight in base pre-treated skin.

TABLE 1. Mast cell and skin histamine levels after 6 weeks' treatment with clobetasol propionate 0·05% and base

Treatment	Mast cells ($n = 4$; 10 HPF; mean ± SE)	Skin histamine ($n = 4$; ng/mg per dry weight; mean ± SE)
Base	19·5 ± 3·84	4·50 ± 1·70
Clobetasol propionate 0·05%	1·75 ± 1·18	1·10 ± 0·47

TABLE 2. Flare reactions to intradermal compound 48/80 and histamine at 6 weeks (cm^2) in two patients in base- and steroid-treated skin

	Flare area (cm^2)							
	48/80 (0·1 μg/0·05 ml)		48/80 (1·0 μg/0·05 ml)		48/80 (10·0 μg/0·05 ml)		Histamine (0·1 μg/0·05 ml)	
Subject	Base	Steroid	Base	Steroid	Base	Steroid	Base	Steroid
A	8·5	0·5	12·0	0·8	15·0	6·08	11·5	8·0
B	1·33	0·0	0·8	0·5	3·2	0·5	1·08	1·0

Flare area after intradermal compound 48/80 and histamine

After intradermal (i.d.) injections of compound 48/80 in base pretreated skin there was a dose-related increase in flare area in one of two patients studied. Flare areas were 8·5, 12·0 and 15 cm^2 respectively for 0·1, 1 and 10 μg of compound 48/80. These areas were markedly decreased in steroid pretreated skin in this patient, (0·5, 0·8 and 6·08 cm^2 for 0·1, 1 and 10 μg compound 48/80 respectively). A dose-related increase in flare areas after i.d. compound 48/80 was not striking in the second patient. However, this patient also demonstrated a decrease in flare area after i.d. compound 48/80 in steroid pretreated skin. (1·33, 0·8 and 3·2 cm^2 in base pretreated and 0·0, 0·5 and 0·5 cm^2 in steroid pretreated areas for 0·1, 1·0 and 10·0 μg compound 48/80 respectively) (Table 2). The erythemal reaction to histamine did not appear to be diminished by steroid treatment. In both patients flare areas after i.d. injection of histamine 0·1 μg were 11·5 and 1·08 cm^2 in base pretreated, and 8·0 and 1·0 cm^2, in steroid pretreated skin.

DISCUSSION

This study shows that long-term application of a potent topical corticosteroid in patients with symptomatic dermographism decreases wealing in response to stroking. Mast cell numbers, skin histamine levels and flare reactions following the i.d. injection of the mast cell degranulating agent compound 48/80 are also reduced.

Most normal people will weal if sufficient pressure is exerted,[8,12] but in patients with SD the wealing occurs at a lower pressure threshold and is associated with itching. A pressure of $3{\cdot}5 \times 10^5$ Pa (35·7 g/mm^2)[7,8] or less will reproduce the response in patients with SD when using the dermographometer.

In SD lesional skin the mast cell granules become swollen and electronlucent[13] and degranulation of mast cells is associated with an increase in the level of plasma histamine.[1] The levels of histamine were raised in dermal perfusates from dermographic patients[14] and histamine may be the primary mediator involved in the wealing mechanism, although one report implicated kinins.[15]

The effect of topical betamethasone 0·05% under 8 h occlusion daily for 6 weeks has been studied in patients with urticaria pigmentosa (UP).[4] Pruritus and Dariers's sign were absent 6 weeks later. Hyperpigmentation and the extent of skin mastocytosis as assessed visually had decreased by 12 weeks. The skin histamine level in one patient had decreased after 6 weeks' treatment and remained lower, at 12 weeks, than pretreatment levels. Light microscopy and transmission electron microscopy revealed an absence of recognizible mast cells at the end of 6 weeks and up to 24 weeks after treatment had been stopped. Our results differ in that mast cells were found 24 weeks after treatment had been stopped in the two patients who returned for further biopsies. The effect of long-term application of topical steroid on mast cells is either to make them unrecognizable or to cause their removal from skin. Changes observed after 3 weeks application of topical corticosteroid[2] were similar to those seen in dying cells.

We have demonstrated an association between the clinical, morphological and pharmacological findings after topical corticosteroid application in SD. Taken in conjunction with the response of SD to antihistamines[7] this association supports the view that release of histamine from mast cells is of primary importance in the production of wealing in SD. The decrease in flare areas evoked by intradermal injection of compound 48/80, observed when the numbers of mast cells are reduced is presumably due to the dependence of the cutaneous reaction to compound 48/80 on release of mediators from degranulated mast cells. Corticosteroid-induced reduced reactivity of the skin vasculature is unlikely to be a contributory factor to reduced wealing following stroking and intradermal injection, since histamine did not produce a constantly reduced erythematous flare in steroid-treated skin compared with base-treated skin.

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Failure of terfenadine in relieving the pruritus of atopic dermatitis

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SUMMARY

We report the results of a double-blind, placebo-controlled, cross-over study of terfenadine 120 mg twice daily for pruritus in atopic dermatitis. Twenty-eight subjects with chronic atopic dermatitis received both terfenadine 120 mg b.d. and placebo each for a period of 1 week. Response was recorded by the subjects using visual analogue scales for severity of pruritus twice daily for the last 4 days of each treatment phase. There was no benefit from terfenadine.

Pruritus is usually the most distressing feature of atopic dermatitis. Antihistamines have long been prescribed in the belief that they reduce pruritus by blocking the action of histamine in the skin. It is also recognized that the sedative action of many antihistamines is of considerable value in itchy dermatoses.[1] A persisting controversy regarding the role of antihistamines in the treatment of atopic dermatitis is the relative importance of sedation and peripheral histamine-receptor blockade. The evaluation of non-sedative antihistamines is one method of resolving this controversy. We report a double-blind, placebo-controlled, cross-over study of terfenadine for relieving pruritus in atopic dermatitis.

METHODS

Twenty-eight subjects, aged 11–67 (mean age 28·6 years), suffering from atopic dermatitis, as defined by the criteria of Hanifin and Rajka,[2] were enrolled in the study. All subjects gave signed informed consent to participation in the trial.

Each subject received terfenadine 120 mg twice daily for 1 week and matched placebo for a week in randomized order. To allow for wash-out of the antihistamine, assessment was performed only on the last 4 days of each treatment. All subjects were receiving topical steroids and emollients sufficient to maintain their dermatitis in a stable condition. No changes to therapy were made during the 4 weeks prior to the study and patients were asked not to alter other aspects of their treatment during the study.

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Subjects were asked to indicate the severity of their pruritus over the preceding 12 h on a visual linear analogue scale twice daily, at 08·00 h and at 20·00 h for the last 4 days on each treatment. Absence of pruritus scored zero and the most severe pruritus ever experienced scored 10. In addition, subjects were examined at the end of each treatment period and scored 0–4 for the severity of excoriation present over the entire skin surface. All clinical assessments were performed by the same observer. Subjects were questioned about side-effects and specifically asked about drowsiness at the end of each treatment period.

RESULTS

Twenty-four patients completed the study having complied precisely with the protocol. Four subjects were eliminated from the analysis due to failure to comply with the protocol, either by changing topical steroid treatment, or by failing to take the trial medication as directed.

Response to treatment

The visual analogue scores were summated and the two treatment phases compared using the Wilcoxon signed-rank matched-pairs test. There was no evidence of any difference between terfenadine and placebo. The mean scores (and standard errors of the means) were 23·95 (±4·9) for the terfenadine phase and 25·13 (±5·1) for the placebo phase. In 13 subjects pruritus scores were higher on placebo, and in 11 subjects higher on terfenadine. There was no evidence of carry-over or period-effect when pruritus scores were assessed using the method of Hills and Armitage.[3]

The clinical assessment of the severity of excoriation remained the same between treatment phases in 16 patients. Three patients scored one point better on terfenadine, and five scored one point better on placebo.

Side-effects

One patient complained of drowsiness on placebo and one on terfenadine. No other side-effects were reported.

DISCUSSION

There is some evidence that histamine plays a role in the pruritus of atopic dermatitis. Basophil infiltration in response to a mild abrasion in contact with house dust mite allergen has been demonstrated.[4] It has also been claimed that the histamine levels in both plasma[5] and skin[6] may be elevated.

The data from investigations of the efficacy of antihistamines in atopic dermatitis are far from conclusive. An improvement in pruritus was demonstrated in a study using hydroxyzine, which has a sedative action[7] but this trial was open. In a double-blind, parallel study in children, hydroxyzine improved pruritus significantly better than cyproheptadine.[8] A double-blind, placebo-controlled, cross-over study could demonstrate no response to chlorpheniramine, cimetidine, or a combination of both these agents,[9] and a double-blind, placebo-controlled, cross-over study of a non-sedative antihistamine (LN2974) in atopic dermatitis, was unable to demonstrate any benefit.[10] However in contrast, a recent placebo-controlled, double-blind, parallel study of the non-sedating antihistamines acrivastine and terfenadine, has demonstrated a slight but clear benefit from each drug.

We believe that the cross-over format, used in our study and that of Savin *et al.*[10] is to be preferred to the parallel design as used by Doherty *et al.*[11] because of the inevitable variation between subjects, both in disease severity and the use of visual analogue scales. It was decided to use a high dose of terfenadine to ensure that any response obtained would be maximal. We considered that a 3 day wash-out period was adequate because the therapeutic effect of terfenadine is generally not considered to last beyond 3 days after an oral dose.[12,13]

The results of our study provide no support for the use of terfenadine in atopic dermatitis, nor for a significant role for histamine in the pruritus of atopic dermatitis. We accept that there are always inherent difficulties in the measurement of pruritus and the methods available have limited sensitivity but there was no suggestion of any response to terfenadine.

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Azathioprine treatment in chronic actinic dermatitis: a double-blind controlled trial with monitoring of exposure to ultraviolet radiation

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SUMMARY

Oral azathioprine was compared with placebo in a double-blind controlled trial of therapy in chronic actinic dermatitis (CAD), a rare eczematous photodermatosis. Eighteen severely affected patients were randomly allocated to azathioprine 50 mg t.d.s. or placebo over a 2-year period. Severity of itch and rash were assessed weekly by each patient on a visual analogue scale and overall clinical status monthly by a medical observer. Monitoring of patient ultraviolet radiation (UVR) exposure was undertaken throughout treatment by polysulphone film lapel-badge dosimetry. Five of 8 patients treated with azathioprine but none of 10 placebo patients achieved remission within 6 months. One patient could not tolerate treatment because of gastrointestinal effects. No haematological or hepatic abnormality was noted. The marked improvement in clinical status of actively treated patients ($P < 0{\cdot}02$, Fisher's exact test), led to early termination of the trial. Oral azathioprine therapy is an effective and usually well tolerated treatment in chronic actinic dermatitis.

Chronic actinic dermatitis (CAD) is a rare photosensitive disorder usually affecting elderly men.[1] The clinical picture ranges from eczema of light-exposed areas with ultraviolet (UV) B sensitivity only, or photosensitive eczema,[2] to marked lymphoma-like induration and infiltration of the same areas with UVB and UVA photosensitivity, or actinic reticuloid.[3] Erythroderma may also occur. Its histology varies from a chronic eczema appearance to a dense dermal infiltrate showing epidermotropism with some cellular atypia resembling mycosis fungoides. The condition is usually severe and refractory to treatment. Other names applied to this and

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clinically similar disorders include 'persistent light reaction' (PLR)[4] and 'the photosensitivity dermatitis and actinic reticuloid syndrome' (PD/AR).[5] PLR refers to patients with persistent photosensitivity secondary to previously discontinued exposure to a drug or chemical, while PD/AR is now used synonymously with CAD and refers to light-sensitive patients who often demonstrate contact allergic reactions to photoactive airborne allergens such as fragrances[6] and oleoresins.[7,8] Thus, PLR and CAD (PD/AR) are associated with similar clinical pictures, but CAD excludes disease with associated known primary aetiological agents, such as drugs or chemicals. Patients with CAD may have increased contact susceptibility to exogenous allergens, but these do not appear of prime importance in inducing the persisting light sensitivity of the skin.

CAD is usually longstanding, but spontaneous improvement may rarely occur.[1] While confinement to a dark or UVR-shielded room usually leads to improvement, relapse may occur on re-exposure even to fluorescent lighting although tungsten bulbs are generally safe. Sunscreens, topical steroids and high dose systemic steroids may be partially effective, but rarely adequately suppress the disease. Immunosuppressive therapy has therefore been tried and was reported effective in anecdotal observation[9] and a later retrospective assessment,[10] both suggesting favourable results with azathioprine. We have assessed the benefit of this treatment in a double-blind controlled trial with continuous monitoring of patient UVR exposure to ensure equivalent exposure for both treatment groups.

METHODS

An average of only about two new cases of CAD per month are referred to St John's Hospital for Skin Diseases, London and some, for reasons of distance, health, age, or results of investigations were not eligible for the study. Only one patient per month was eventually available for the trial, and this necessitated a study to allow addition of patients as they became available throughout the year, to enable sufficient accumulation of patients over a 2–3-year period for the study.

Patients were required to wear polysulphone film lapel-badges as in previous trials to ensure equivalent ultraviolet exposure of the treatment groups by monthly spectrophotometric measurement of the change in absorbance of polysulphone at 330 nm[11] and to permit a valid comparison of the groups.[12-14] Patients wore and changed the badges weekly throughout the trial, keeping those not in use refrigerated in a dark container and returning them monthly.

Patients with a history of gastrointestinal problems such as gastritis, peptic ulceration or hiatus hernia, and of liver disease or renal impairment were excluded, as were elderly patients with forgetfulness or inability to comprehend the protocol of the trial. All were investigated prior to trial entry by irradiation monochromator testing on the skin of the back[15] to confirm diagnosis and document the degree of photosensitivity. Patch and photopatch tests excluded patients with an allergic contact or photocontact dermatitis.

Informed consent was obtained from all patients. Eighteen consecutive severely affected patients were randomly allocated to azathioprine 50 mg t.d.s. or placebo therapy over a 2-year period. Patients documented the severity of itching and rash weekly on a 10-cm visual analogue scale and were assessed clinically on a monthly basis by a supervising clinician, the extent of rash being documented according to the rule of nines.[16] Each month, haemoglobin, white cell and differential count, platelet count, erythrocyte sedimentation rate, liver function and renal function were assessed, side-effects from the medication documented, visual analogue scale markings looked at to ensure correctness and patients' use of polysulphone film badges carefully checked; the previous month's visual analogue scales and accompanying film badges were then collected and the following month's supply dispensed. Patients were considered to be in remission when they were free of itch and had no eczematous changes.

Statistical methods

Since up to twelve repeated monthly observations were made on patients, it was necessary to take into account within- and between-patient variation in itch and rash levels together with differences in UV exposure, analysis being carried out using the GLIM statistical package.[17] Analysis of the effect of treatment on the two groups was carried out at 1, 3 and 6 months after the start of treatment using unpaired *t*-tests, and of the overall clinical outcome comparing active and placebo groups using Fisher's exact test.

RESULTS

The clinical features of the active- and placebo-treated groups are shown in Table 1. Six males and two females (mean age 64 years) received active therapy and 10 men (mean age 66 years) received placebo therapy. The two groups were similar with regard to most clinical characteristics, but patients on active treatment had more extensive involvement of the skin than those on placebo (Table 1), and all eight patients were both UVB and UVA sensitive as measured by monochromator test reactions compared with six of the ten placebo-treated patients at the start of the trial (Table 2). Patients with UVB and UVA photosensitivity are usually more severely affected clinically and more difficult to manage than patients affected by UVB alone. The mean duration of treatment was 8·4 months (range 1·5–12 months; active group) and 4·1 months (range 0·1–11 months, placebo group). Although Table 1 shows that the mean duration of treatment was greater in the active group, this was due to one placebo patient dropping out after only 3 days. Five of the eight active patients were treated for at least 5 months compared with five out of 10 placebo patients (Fig. 1).

Mean monthly UV exposure was greater only by 32·5 J/cm^2 (SE 144·2) in the active group compared with placebo, and we could not show that this had a material effect on response to treatment in this relatively small sample of patients. A decrease of around 0·7 units on both the itch and rash scale was associated with an increase of 1000 mJ exposure in the active group. This increase in exposure among the controls led to an increase of 0·6 units on the itch scale and 0·08 units on the rash scale. This difference in increase was 1·28 units (95% confidence interval: 00·09, 2·47) in itch and 0·78 units (95% confidence interval: 0·34, 1·90) in rash, thus only in the control group was a positive relationship observed between increasing UVR exposure and symptoms of itch and rash.

TABLE 1. Clinical characteristics of azathioprine and placebo groups and length of time spent in the trial (ranges in parentheses)

	Azathioprine group	Placebo group
Mean age (years)	64 (48–75)	66 (48–84)
Sex (M, F)	6 M, 2 F	10 M
Mean surface area affected at start of trial (%)	36·2 (6–90)	30·9 (5–95)
Mean duration of treatment (months)	8·4 (1·5–12)	4·1 (0·1–11)

TABLE 2. Spectral sensitivity of patients in azathioprine and placebo groups. The azathioprine group was more severely photosensitive compared with controls

	Wavebands (nm)	Number of patients
Azathioprine group		
Reduced MED	300–340	3
	300–360	5
Placebo group		
Reduced MED	300–307·5	1
	300–320	3
	300–340	4
	300–360	2

Five of eight azathioprine-treated patients achieved remission (defined as absence of rash and itching), one improved (as assessed by the clinician's documentation of extent of rash and by the visual analogue scales for rash and itch) within 3–12 months (Figs 2, 3 and 4), one did not improve over 8 weeks and one defaulted at 6 weeks with gastrointestinal symptoms. None of 10 placebo patients improved, although one defaulted within days of starting the trial and two were only in the trial for 1 month. The difference between active and placebo groups in symptoms of itch and rash on the visual analogue scales and in extent of rash as documented by supervising clinicians according to the rule of nines and as assessed by unpaired t-tests at months 1, 3 and 6 is summarized in Table 3. Beneficial changes were significantly greater in the active group over 1, 3 and 6 months. Further analysis of the intervening months showed similar results although for the sake of brevity this data is not shown. The difference in probability of remission for active versus placebo treated patients (Table 4) was significant, ($P < 0{\cdot}02$, Fisher's exact test).

The significantly better clinical status of some of the patients which was apparent prior to breaking the code for the trial, led us to terminate the trial earlier than expected for ethical reasons. We suspected that some of the patients that were doing badly were on placebo therapy but this was not confirmed until the code was broken. Side-effects encountered in the trial were

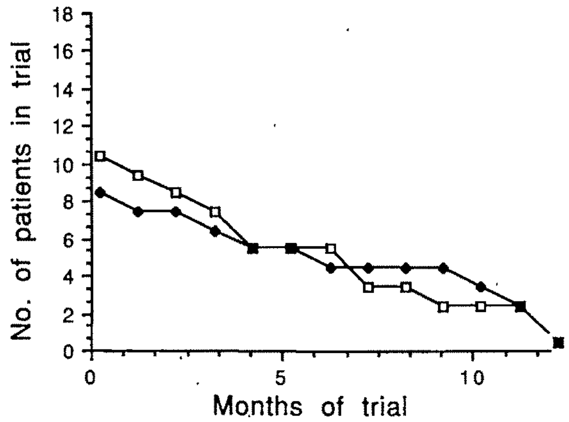

FIGURE 1. Duration in trial of patients on placebo, □——□ and active ◆——◆ treatment.

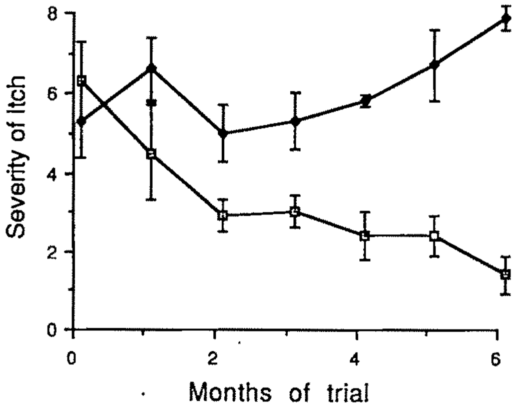

FIGURE 2. Relative severity of itch as documented by visual analogue scale (VAS) in active □——□ and placebo ◆——◆ groups for the 6 months included in the analysis. Error bars represent standard error of the mean (SEM).

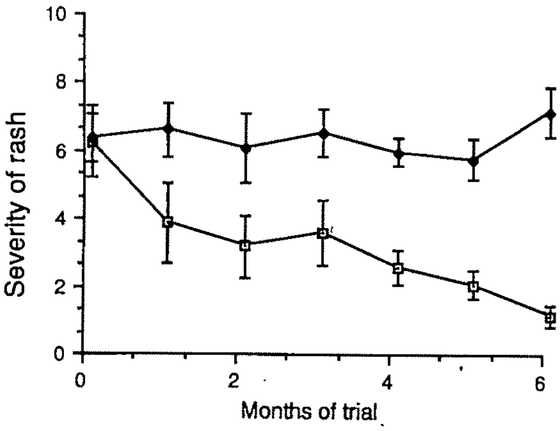

FIGURE 3. Relative severity of rash (± SEM) as documented by visual analogue scale (VAS) in active □——□ and placebo ◆——◆ groups for the 6 months included in the analysis.

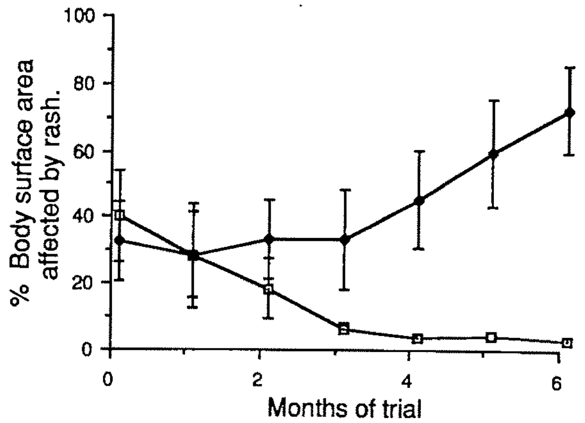

FIGURE 4. Extent of rash (± SEM) as documented using the rule of nines throughout the 6 months included in the analysis. Active □——□, placebo ◆——◆. Error bars are not visible for months 3–6 in the azathioprine group as the figures were very small.

TABLE 3. Analysis of symptoms of rash, itch and extent of rash comparing active and placebo groups using unpaired *t*-tests

Month	No. of Patients		Mean reduction in itch score			Mean reduction in rash score			Mean reduction in extent of rash		
	Azathioprine	Placebo	Azathioprine	Placebo	95% confidence interval for difference	Azathioprine	Placebo	95% confidence interval for difference	Azathioprine	Placebo	95% confidence interval for difference
0–1	7	9	1·8	−1·3	(0·3, 5·8)*	2·4	−0·2	(0·5, 4·6)*	12·2	4·3	(−4·0, 28·5)
0–3	6	7	3·6	−0·6	(1·3, 7·0)*	3·1	−0·2	(0·6, 5·9)*	39·2	4·0	(4·5, 65·8)*
0–6	4	5	4·6	−3·5	(3·8, 12·5)*	4·9	−1·1	(1·7, 10·2)*	34·4	−27·8	(11·8, 112·5)*

* $P < 0·05$

TABLE 4. Clinical state of patients at the time the trial was terminated. The difference in outcome between the two groups was significant ($P < 0{\cdot}02$) Fisher's exact test

	Azathioprine	Placebo
Patients (no.)		
Remission	5	0
Improved	1	0
No change	2	6
Deteriorated		4

few and there were no biochemical or haematological abnormalities. Only one patient discontinued treatment because of gastrointestinal intolerance due to azathioprine.

DISCUSSION

This study shows azathioprine to be a well tolerated and effective treatment in the previously virtually intractable condition of CAD. It appears to be a valuable treatment for severely affected patients. The mode of action of azathioprine in CAD is, however, uncertain. It is slowly metabolized into 6-mercaptopurine and the active intracellular metabolite, 6-thioguanine nucleotide, is thought to be responsible for its immunosuppressive action.[18,19] When naturally occurring purine bases in DNA are replaced by purine analogues the sensitivity of cells to UV and ionizing radiation increases: replacement of guanine by thioguanine in *Escherichia coli* DNA makes the cell more sensitive to killing by UVR, especially 320–360 nm.[20–22] It seems possible that azathioprine acts on the white cells in the bone marrow, as well as on Langerhans cells in the dermis and epidermis which could be preferentially affected by the augmentation of the reaction by UV irradiation. Animal studies have shown that topical application of azathioprine leads to reduction of Langerhans cell numbers in mice,[23] and PUVA, another treatment modality that suppresses Langerhans cell numbers,[24] is also apparently effective in CAD.[25] It is also possible that long-term administration of azathioprine leads eventually to cutaneous depletion of Langerhans cells by the marrow suppressive effect.

There has been much speculation on the nature of the underlying defect in actinic reticuloid: whether there is a cell membrane defect and increased susceptibility to the effects of UV light.[26] If so, it is conceivable that CAD could be caused by continuing antigen presentation from damaged cell membranes, leading to the persistent eczematous response. Thus, if azathioprine exerted its effects by depletion of Langerhans cells, antigen presentation, whether appropriate (from damaged cell membranes) or even inappropriate (because of an intrinsic abnormality of the Langerhans cell), would be reduced with a consequently muted immune response. Alternatively azathioprine may exert its effects by a non-specific anti-inflammatory effect, since it is also effective even in non-photosensitive eczematous disorders as a steroid sparing agent.[9]

Provided patients are carefully selected and those with haematological, liver or renal abnormalities and gastrointestinal problems excluded azathioprine appears to be well tolerated in the treatment of CAD. The risk of lymphoreticular and cutaneous malignancy[27] from long-term azathioprine administration is likely to be small since most patients are elderly and short-term administration of the drug (6 months) appears sufficient to induce remission in the majority of patients. For patients with severe persistent disabling CAD in whom reasonable care in restricting light exposure, regular application of topical sunscreens and frequent use of topical or occasional use of oral corticosteroid therapy have been ineffective, we recommend a trial of azathioprine therapy until remission is achieved or for a maximum period of 6 months.

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Hydroxyurea in the management of therapy resistant psoriasis

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SUMMARY

Eighty-five patients with extensive chronic plaque psoriasis, unresponsive to conventional topical therapy, were treated with long-term hydroxyurea in a dose of 0·5–1·5 g daily. Fifty-two (61%) had a satisfactory remission during treatment without significant adverse effects. Treatment was discontinued in 33 patients (39%), due to an inadequate response or significant relapse during treatment and because of adverse reactions (19%). Four (4·7%) patients on hydroxyurea developed actinic psoriasis. Significant haematological abnormalities occurred in 30 patients (35%), but these became normal following a reduction in the dose of hydroxyurea or temporarily stopping the drug. In only six was it considered necessary to discontinue treatment because of bone marrow suppression. Our experience suggests that hydroxyurea is an effective long-term treatment for psoriasis that is refractory to conventional topical therapy and that the incidence of serious adverse effects compares favourably with other cytotoxic drugs.

Hydroxyurea is an antimetabolite which is widely used in the treatment of a variety of neoplastic disorders, principally chronic myeloid leukaemia, advanced ovarian carcinoma and tumours of the head and neck, either as a single agent or in combination with radiotherapy.[1–6] It has also been shown to be an effective treatment for recalcitrant psoriasis in the short-term,[7] as continuous therapy for up to 28 weeks[8] and intermittent treatment for up to 18 months.[9] There have been few accounts of experience with hydroxyurea in the longer term treatment of resistant psoriasis.[10] Hydroxyurea is not now as widely used by dermatologists, possibly because of the concern as to its potential for bone marrow suppression.[8,11]

We have found hydroxyurea a safe and effective long-term treatment for resistant psoriasis, provided that there is adequate haematological monitoring. In this study we have reviewed all the patients treated with hydroxyurea in our department over the last 8 years.

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METHODS

Between 1980 and 1988, 85 patients with psoriasis were treated in our department with hydroxyurea (46 males, 39 females; mean age 56 years, age range 25–79 years). The mean duration of their disease was 13 years. All had severe widespread chronic plaque psoriasis, refractory to conventional topical therapy.

Hydroxyurea was avoided where possible in women of child bearing age, but if considered necessary, was administered only when adequate contraception could be assured. Subject to satisfactory full blood count and biochemical assessments of hepatic and renal function, hydroxyurea was usually commenced at a dose of 1·5 g daily. The dose was adjusted according to clinical response and evidence of bone marrow suppression was determined by 2–4 weekly blood counts. A maintenance dose of 0·5–1·5 g daily was achieved in the majority of patients. Where full remission occurred and was maintained for several months, the dose of hydroxyurea was gradually reduced and if possible the drug was stopped. Periodic biochemical assessments of renal and hepatic function were made throughout treatment at 3–6 monthly intervals.

The response to therapy and adverse effects experienced were recorded in all patients, and 52 patients currently on treatment were investigated for evidence of long-term toxicity. In these patients their liver function tests, serum urea and electrolytes, creatinine clearance, serum urate, thyroid function tests, serum vitamin B_{12}, red cell folate and an autoantibody screen were determined.

TABLE 1. Response to therapy (85 patients were included in the study)

Response to therapy	Number of patients (%)
Good (complete to near complete clearing)	51 (60)
Good but relapse on treatment	3 (3·5)
Moderate (clearing such that residual lesions could be managed at home)	17 (20)
Poor	14 (16)

TABLE 2. Number of adverse effects (85 patients were included in the study)

Adverse effects encountered	Number of patients (%)
None	48 (57)
Adverse effects encountered	37 (43)
Continuing therapy despite adverse effects	3 (3·5)
Continue on reduced dose with no adverse effects	18 (21)
Treatment stopped as a result of adverse effects	16 (18)

RESULTS

Response to treatment

The results of treatment of psoriasis with hydroxyurea in the 85 patients are summarized in Tables 1 and 2. Sixty-eight (80%) had a good or moderate response, in 51 (60%) there was a complete or almost complete clearance of lesions and in 17 (20%) there was a partial clearance. Fifty-two patients (61%) were currently taking hydroxyurea and the mean duration of treatment was 16 months (range 3–96 months). In 17 patients (20%) hydroxyurea was stopped as a result of an inadequate response to therapy. Three patients (3·5%) had a good initial improvement during the first 4–6 weeks of therapy but subsequently relapsed. Eight patients (9·4%) had moderate clearing of lesions but residual lesions could not be satisfactorily controlled by topical measures, and six patients (7%) had little or no clearing of lesions. Of the 17 patients who had an inadequate response to hydroxyurea, five have been subsequently controlled on methotrexate, three on PUVA, four on etretinate and five on a combination of PUVA and etretinate. None of the patients studied had a long-term remission of their psoriasis after stopping hydroxyurea. Only one patient (6%) experienced an acute flare of psoriasis on stopping therapy, the other having a gradual deterioration.

Adverse effects

Adverse effects which may have resulted from therapy with hydroxyurea were observed in 37 patients (43%) and the details of these effects are given in Table 3. In 16 patients (18·8%) the adverse effects necessitated withdrawal of the drug. In all but two cases who had swelling of the legs there was a full recovery following withdrawal of hydroxyurea.

Haematological adverse effects. Some reduction in the various haematological values were noted in many of the patients on treatment. However, clinically significant changes only occurred in 30 patients (35%). The more frequent adverse effects were anaemia (Hb < 11 g/dl), leucopenia (counts $< 3{\cdot}5 \times 10^9$/l) and thrombocytopenia (counts $< 150 \times 10^9$/l) either alone or in combination. An isolated asymptomatic reduction in haemoglobin was observed in 10 patients (11·8%), (mean 10 g/dl, minimum 8·8 g/dl). In seven this was considered to be a result of bone marrow suppression and the haemoglobin level became normal on reducing the dose of hydroxyurea. In the remaining three patients (3·5%) the anaemia was associated with reduced vitamin B_{12}, folate or iron deficiency respectively and was corrected by the appropriate replacement therapy without a change in the dose of hydroxyurea. Isolated asymptomatic reductions in leucocyte counts were observed in six patients (7%) (mean $2{\cdot}6 \times 10^9$/l, minimum $1{\cdot}8 \times 10^9$/l) and represented reductions chiefly of polymorphonuclear leucocytes. These abnormalities were corrected within 6–8 weeks by reducing the dose of hydroxyurea or by temporary interruption of treatment. Reintroduction at a lower dose caused no problems in four subjects. However, in the remaining two patients reintroduction of hydroxyurea resulted in further reductions in the leucocyte count and therapy was discontinued permanently. Modest isolated asymptomatic thrombocytopenia was observed in two patients (2·3%) (mean 90×10^9/l, minimum 76×10^9/l) and was corrected within 8 weeks by a reduction in hydroxyurea dosage.

Combined leucopenia and anaemia with or without thrombocytopenia was encountered in 10 patients (12%) and three patients had a symptomatic anaemia that required a single blood transfusion. The abnormalities were otherwise asymptomatic and became normal within 6–8 weeks of discontinuing hydroxyurea. In six subjects, hydroxyurea was restarted at a lower dose without further problems. In the remaining four patients, hydroxyurea was not restarted. In the

TABLE 3. Adverse effects encountered on hydroxyurea

	No. of patients affected (%)	No. of patients stopping Rx. (%)
Haematological		
Anaemia	10 (11·7)	0
Leucopenia	6 (7)	2 (2·3)
Thrombocytopenia	2 (2·3)	0
Pancytopenia	10 (11·7)	4 (4·7)
Folate deficiency	2 (2·3)	0
B_{12} deficiency	2 (2·3)	0
Low serum iron	1 (1)	0
Pigmentation		
Diffuse	4 (4·7)	0
Localized to plaques	5 (5·8)	0
Actinic psoriasis	4 (4·7)	0
Alopecia	3 (3·5)	1 (1)
Gastrointestinal		
Dyspepsia	1 (1)	0
Nausea	2 (2·3)	1 (1)
Hepatic		
Raised hepatic alkaline phosphatase	2 (2·3)	2 (2·3)
Raised SGOT, SGPT and alkaline phosphate	1 (1)	1 (1)
Autoimmune		
Thyroiditis	1 (1)	1 (1)
Diabetes mellitus	1 (1)	1 (1)
Depression	2 (2·3)	1 (1)
Leg swelling	2 (2·3)	2 (2·3)
Infections	1 (1)	0

majority of cases, the changes in haematological variables were discovered early on in routine testing and progressed gradually if no alteration in the dose of hydroxyurea was made. Seven patients (8%) developed gradual reductions after several months of stability while on a constant dose of hydroxyurea. In five patients (5·8%) there was a rapid reduction in blood counts over a period of 8 weeks, and these cases suggest the possibility of either poor compliance with therapy or other unidentified external factors to produce bone marrow suppression. A macrocytosis was observed in all patients receiving hydroxyurea. In two this was associated with a significant low serum vitamin B_{12} and in a further two with a reduction in the serum folic acid. One each of these had a mild anaemia, the others were asymptomatic. The macrocytosis induced by hydroxyurea is reversible, but our impression is that it can persist for some months. Although we were unable to assess the macrocytosis in all patients after they had discontinued hydroxyurea, the level was still raised at 3, 4 and 6 months respectively post-treatment in three patients.

Pigmentation. Four patients (4·7%) developed a diffuse hyperpigmentation of lesional and non-lesional skin in both sun-exposed and non-sun-exposed sites within 3–6 months of starting hydroxyurea. The pigmentation was of no concern to patients and usually persisted. Five patients (5·8%) developed pigmentation in the areas of resolving plaques, that gradually faded and was probably post-inflammatory.

Actinic psoriasis. Four patients (4·7%) demonstrated a flare of their psoriasis in the light-exposed areas while on treatment with hydroxyurea.

Alopecia. Three patients (3·5%) developed a moderate diffuse alopecia. In one hydroxyurea was discontinued with subsequent normal hair growth. The remaining two patients continued on hydroxyurea at a lower dose with full regrowth of hair.

Gastrointestinal. Three patients (3·5%) developed gastrointestinal symptoms. One patient developed dyspepsia and a barium meal revealed a duodenal ulcer which responded to conservative treatment without a change in the dose of hydroxyurea. Two patients developed nausea that settled with a reduction in dose in one but the drug was stopped in the other.

Hepatic. An elevation of liver enzymes was observed in three patients. One patient had an asymptomatic rise in alkaline phosphatase noted prior to therapy which remained unchanged on treatment. The second patient developed an isolated elevation of hepatic alkaline phosphatase after 1 month on hydroxyurea. Liver biopsy was normal and the level returned to normal when treatment was discontinued. A third patient was found to have an asymptomatic elevation of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. Liver biopsy revealed changes of mild hepatitis. Hepatic enzyme levels returned to normal within 2 months of discontinuing hydroxyurea.

Autoimmune disorders. A 61-year-old female patient with a past history of lichen sclerosus et atrophicus with known thyroid microsomal antibodies (titre 1:81) prior to hydroxyurea therapy, developed clinical thyroiditis and hypothyroidism 2 weeks after starting hydroxyurea at a dose of 1·5 g daily. Hydroxyurea was withdrawn and the thyroiditis settled over 16 weeks. The hyperthyroidism subsequently persisted and was corrected by thyroxine replacement therapy.

A 38-year-old male patient who was well prior to hydroxyurea therapy, developed diabetic ketoacidosis after 5 months treatment at a dose of 1·5 g daily. Hydroxyurea was continued and the diabetes controlled with insulin.

Psychological effects. Two patients had marked depression during treatment, one of whom had a past history of depression. The second patient was a widower who had been a frequent attender for daily out-patient treatment of his psoriasis. Treatment with hydroxyurea resulted in considerable clearing of his lesions and his out-patient attendance was reduced from daily to monthly. He became increasingly depressed, probably as a result of his social isolation, and subsequently committed suicide.

Leg swelling. Two patients described an asymptomatic, symmetrical swelling of the lower legs, which spared the dorsum of the feet and which developed within 6 weeks of commencing hydroxyurea. There was a non-pitting swelling with no skin changes and suggestive of increased subcutaneous fat. These appearances persisted unchanged after withdrawal of hydroxyurea.

Infections. No serious infections were encountered in any of the patients included in the study. One 52-year-old female had an episode of bronchitis while on hydroxyurea. This infection responded to appropriate therapy without any reduction in hydroxyurea.

DISCUSSION

Hydroxyurea has been shown to be effective in the treatment of resistant psoriasis, when given continuously[7,8] and intermittently.[9] It inhibits cell proliferation by interfering with the synthesis of DNA[1] and affects the replication of cells in the epidermis.[12] Our findings show that hydroxyurea is an effective agent in the long- and short-term treatment of recalcitrant psoriasis. In common with previous reports, we found that relapse after withdrawal of hydroxyurea was frequent,[9] but acute relapse occurred in only one of our patients. Relapse occurs following cessation of treatment with the other agents used for refractory psoriasis and probably reflects the pattern of disease in these patients. As with other modes of treatment for resistant psoriasis we found that adverse effects were not infrequent. The major concern expressed about hydroxyurea therapy is the frequency of bone marrow suppression.[8,11] Whilst a fall in the blood count was usual, levels below the normal range were less common, and in our patients were reversed on reducing the dose of hydroxyurea. In a small number of patients hydroxyurea apparently unmasked what was probably a pre-existing iron, folate or vitamin B_{12} deficiency.

Hydroxyurea induces a megaloblastic erythropoiesis in all patients and this occurs as early as 24 h after the start of treatment.[13] Reports suggest this abnormality resolves quickly after withdrawal of treatment,[14] but we noted three cases where a megaloblastosis was still evident after 3, 4 and 6 months off therapy. A study of serum vitamin B_{12} and folate states in patients treated with hydroxyurea indicated that the defect is not due to a deficiency in these vitamins.[14]

Alopecia is a recognized complication of treatment with hydroxyurea.[15] The three patients who developed alopecia had full recovery of hair growth on reducing or discontinuing treatment. Generalized pigmentation has been described previously in patients treated with hydroxyurea[8] and was observed in four of our patients.

Four patients demonstrated a flare in their psoriasis while on hydroxyurea therapy, which apparently followed sun exposure and was localized to light-exposed areas. Avoidance of sunlight and sun-screens allowed all but one to continue treatment. This phenomenon has not previously been described although an augmentation of erythema in areas previously irradiated has been noted in three patients treated with hydroxyurea in combination with radiotherapy for solid tumours.[16]

Hepatic dysfunction has only rarely been reported with hydroxyurea[17] and two of our patients had evidence of hepatic dysfunction. In both, the elevated enzymes became normal when treatment was discontinued in one and continued in the other. No evidence of hepatic dysfunction was found in the other patients.

Two patients treated with hydroxyurea developed endocrine dysfunction after starting therapy (one hypothyroidism and one diabetes mellitus). It is possible that hydroxyurea-induced immune modulation was responsible for development of these conditions in predisposed individuals, but they may be coincidental. Autoantibodies were not detected in any of the 52 patients taking hydroxyurea at the time of the study, and thyroid function was normal and no patient had glycosuria.

Two patients had swollen legs in this study and whether hydroxyurea was responsible could not be substantiated. Non-pitting oedema of the legs in association with swelling of the fingers and a localized lichenoid eruption in a patient with chronic myeloid leukaemia taking 3 g of hydroxyurea a day has been described.[15] It is not clear whether this patient had changes similar to ours.

Abnormalities in renal function and elevations in serum uric acid and creatinine have been described.[18] Like other authors who have used hydroxyurea for psoriasis, we found no evidence of this in any patients even after long-term use.

Hydroxyurea is an effective long-term treatment for resistant psoriasis. Despite a high incidence of bone marrow suppression, significant haematological problems are uncommon, provided there is regular monitoring of the blood counts with appropriate regulation of the dose of the drug. The present study indicates that at the doses used to treat psoriasis (< 1·5 g daily) serious long-term adverse effects are rare. In this respect hydroxyurea compares favourably with methotrexate which has a potential for producing irreversible hepatic damage[19] and cyclosporin A with its potential for dose-related nephrotoxicity.[20]

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Cutaneous manifestations of cystic fibrosis: possible role of cryoglobulins

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SUMMARY

A 13-year-old girl with cystic fibrosis (CF), severe lung disease, purpura on the lower extremities and with elevated serum mixed cryoglobulins is described. The possible relationship of the purpura in CF with cryoglobulinaemia is discussed.

Involvement of the skin in cystic fibrosis (CF) is rare but skin lesions have been reported that include a maculopapular rash,[1] erythema nodosum,[2,3] rheumatoid nodules,[4] urticaria,[5] and purpura.[1,4,6–8] The purpura in CF seems to be characteristic with an almost uniform clinical presentation. It has been described as 'hypergammaglobulinaemic purpura' (although not all the patients had hypergammaglobulinaemia) and as 'cutaneous necrotizing vasculitis'.

We report on a patient with CF, purpura and with elevated mixed cryoglobulins which to our knowledge has not previously been described.

CASE REPORT

A 13-year-old Caucasian female was diagnosed as having CF at the age of 6 weeks following pneumonia, failure to thrive and the finding of elevated sweat electrolytes. During her first 10 years of life she was hospitalized on five occasions and during the following 3 years she had six further admissions to hospital and all were due to exacerbations of her pulmonary disease. During the past 3 years she has had various treatments, including: theophylline, metaproterenol, acetylcysteine, tetracycline, dicloxacillin, piperacillin, tobramycin, pancrease and vitamins. Since the age of 12 she has had numerous episodes of macular eruptions on the legs and feet accompanied by mild pruritus or burning sensations and these have usually progressed to purpura over the following 2–3 days and faded after 10–14 days. They appeared to be precipitated by tight shoes or stockings, prolonged standing or sustained effort. There was no apparent association with cold temperature or with pulmonary exacerbations or the use of certain medications. There was no history of arthralgia or Raynaud's phenomenon.

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On recent physical examination, she was a well nourished girl with no acute distress. Multiple palpable purpuric skin lesions were seen on the legs and feet, some being confluent and with diameters ranging from 1 mm to several cm. A brown pigmentation of the skin, residual from previous purpura was also noted. Apart from lung signs and moderate clubbing of the fingers the rest of the physical examination was normal. Laboratory tests showed the following results: Haemoglobin was 12·2 g/dl; white cell count was 9600/mm^3 with 61% neutrophils, 30% lymphocytes and 9% monocytes; platelets were 200000/mm^3. Blood chemistry tests (SMA-12) were normal except for a serum total protein of 8·4 g/dl with 4·0 g/dl albumin. The prothrombin time was 9·4 s; partial thromboplastin time was 26·5 s. HbsAg was negative. Anti-nuclear antibody and antibodies against thyroid, adrenal, parietal cells, smooth muscle and mitochondria were negative. Rheumatoid factor was positive at a titre of 1/320. C3 was 214 mg/dl (normal 90–190 mg/dl), C4 was 48 mg/dl (normal 16–22 mg/dl), CH$_{50}$ was 148 IU/ml (normal 80–200 IU/ml). Immunoglobulins were elevated: IgG 2000 mg/dl (normal 575–1600 mg/dl), IgA 980 mg/dl (normal 70–470 mg/dl), IgM 290 mg/dl (normal 70–210 mg/dl), IgE 240 IU/ml (normal less than 25 IU/ml). Circulating immune complexes were elevated: C1$_q$ binding assay was 66% (normal less than 13%) and Raji cell assay for immune complexes was 240 μg aggregated human gammaglobulin equivalent per ml (normal less than 50 μg). Cryoglobulins were elevated: IgG 2·2 mg/dl (normal less than 0·10 mg/dl), IgA 2·7 mg/dl (normal less than 0·02 mg/dl), IgM 1·6 mg/dl (normal less than 0·05 mg/dl), C3 2·4 mg/dl (normal less than 0·005 mg/dl). Serum protein electrophoresis showed a polyclonal elevation of the immunoglobulins. Urinalysis was normal. Sputum culture grew *Pseudomonas aeruginosa* and *Pseudomonas cepacia*. A chest X-ray showed moderate hyperaeration, peribronchial thickening and diffuse cystic changes. Pulmonary function tests showed severe obstructive lung disease with air trapping. The electrocardiogram was normal.

The child was treated with 20 mg prednisone daily, with gradual reduction of dose to 10 mg every other day, and showed some improvement. However, after 18 months of follow-up she has occasional episodes of purpura.

DISCUSSION

Fifteen patients with CF and purpura, have so far been reported.[1,4,6–8] The purpura in CF seems to be a distinctive syndrome with several characteristics. It occurs late in the course of CF, usually in the second or third decade (mean age was 16 years), with no sex predilection. The lesions appear mainly on the lower extremities (14/15 cases), often preceded by pruritus or a burning sensation. Macular eruptions develop which soon become non-blanching and purpuric. The lesions usually fade within 7–14 days, but recurrent episodes are the rule. Our patient and one of the patients of Soter *et al.*[6] showed a partial response to treatment with corticosteroids. The degree of severity of the lung disease does not always correlate with the purpura, but at least 50% of these patients died within 2 years of its appearance. The purpura does not seem to be associated with colonization with any specific micro-organism or treatment. In 40% of the patients the purpura was associated with arthralgia or arthritis.[1,6–8] Renal involvement was minimal or absent. Histological examination of the skin, in seven patients, showed a vasculitis that involved mainly dermal venules with perivascular polymorphonuclear or mononuclear cell infiltrates, and occasionally focal deposits in the dermis of IgG, IgM and IgA.[1,6–8] The characteristic laboratory findings include elevated immunoglobulins (11 out of 15), normal, elevated or decreased serum complement, elevated circulating immune complex (6 out of 9) and occasionally a positive rheumatoid factor. Elevated serum cryoglobulins were found only in our patient. Cryoglobulins were tested, but not detected, in the serum of four out of the 14 previously reported patients.[4,6] However, two of these were uncharacteristic patients, since they had a single event of transient purpura.

Cryoglobulinaemias have been classified into three major categories: type I, monoclonal cryoglobulinaemia; type II, polyclonal cryoglobulinaemia with a monoclonal component; type III, mixed (polyclonal) cryoglobulinaemia.[9] Cryoglobulinaemia may be secondary to viral, bacterial, fungal or parasitic infections, autoimmune disorders or lymphoproliferative diseases.[10] A common clinical manifestation of mixed cryoglobulinaemia is purpura, which is characteristically intermittent and usually involves the lower extremities. Common laboratory abnormalities of mixed cryoglobulinaemia include elevated immunoglobulins and positive rheumatoid factor.[10–11]

To our knowledge, our patient is the first with cystic fibrosis, purpura, and mixed cryoglobulinaemia to be reported. However, in most of the other cases of purpura in CF, cryoglobulins were not measured. We suggest that in view of the very similar clinical and laboratory findings, cryoglobulinaemia was a possible mechanism for the purpura in some of the patients. Cold-induced purpura is present in about 30% of patients with mixed cryoglobulinaemia.[9] Although this was not a recognizable precipitating factor in our patient, it was reported in three of 15 patients with CF and purpura.[4,6] Cryoglobulins may have been present in other patients with CF as in some cases of Henoch–Schonlein purpura.[12] It is not known whether cryoglobulins affect the lungs in patients with CF but a vasculitis involving lung vessels has been reported in mixed cryoglobulinaemia.[13]

Circulating immune complexes (CIC) have been detected in many diseases characterized by persistent antigen stimulation, including CF.[14] CIC have been found in two thirds of the patients with CF and purpura, which is not essentially different from the occurrence of CIC in other CF patients.[14,15] It is possible that particular immune complexes or cryoglobulins are involved in the pathogenesis of the purpura in CF.

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Isolated lichen planus of the nails associated with primary biliary cirrhosis

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SUMMARY

Lichen planus is a relatively common inflammatory dermatosis with characteristic lesions affecting the skin, nails and mucous membranes. Nail changes are present in approximately 10% of patients and can occasionally occur in the absence of skin involvement. The association of lichen planus with primary biliary cirrhosis is now well recognized. We report the first case of lichen planus confined to the nails in a patient with primary biliary cirrhosis.

CASE REPORT

A 52-year-old woman was referred with a 30-year history of abnormal nail growth. Initially only the nails of the great toes had been affected. Subsequently, all the finger- and toe-nails were involved with permanent loss of the left great toe-nail.

Eleven years previously she had been investigated for lassitude and weight loss and liver function tests demonstrated an early obstructive picture (bilirubin 11 μmol/l [0–17 μmol/l], alkaline phosphatase 384 units/l [45–120 units/l] and γ-glutamyl transferase 139 [0–50 units/l] with normal transaminases). Hepatitis serology, erythrocyte sedimentation rate, serum proteins and biliary tract ultrasound were all normal. Serum antimitochondrial antibodies were present in significant titres. The original liver biopsy showed non-specific changes, but later biopsies confirmed the diagnosis of primary biliary cirrhosis. Subsequently, her liver function tests deteriorated and she became jaundiced. Cholestyramine was administered for the symptomatic relief of her pruritus, but no other specific therapy was given and she did not receive penicillamine.

When first seen in the dermatology clinic she was thin and icteric with numerous spider naevi on the upper trunk. There were no other cutaneous stigmata of chronic liver disease. Examination of the skin and mucous membranes showed no signs of lichen planus. All her nails were abnormal and there was swelling, tenderness and violaceous discoloration around the proximal nail fold. The finger-nails showed longitudinal ridging. The toe-nails showed more advanced changes with pterygium formation and complete loss of the nails of the left great and fourth toes (Fig. 1).

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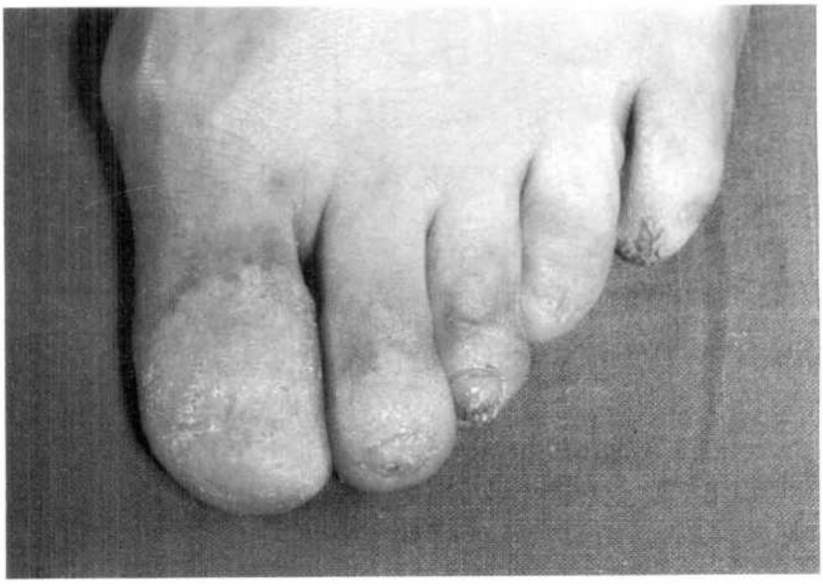

FIGURE 1. Clinical photograph of left foot showing swelling and discoloration adjacent to the proximal nail folds, pterygium formation and complete nail loss.

A Zaias nail biopsy was taken from the left great toe. This demonstrated hyperkeratosis, parakeratosis and acanthosis with a patchy lichenoid chronic inflammatory cell infiltrate that was associated with basal layer liquefaction and cytoid bodies (Fig. 2).

In view of the nail biopsy histology the diagnosis of lichen planus confined to the nails in association with primary biliary cirrhosis was made. Treatment with potent topical steroids

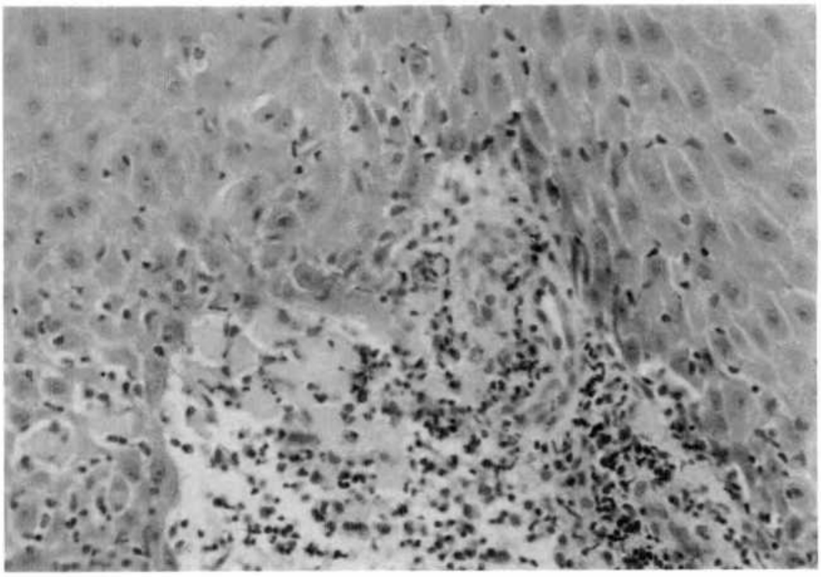

FIGURE 2. Photomicrograph of Zaias nail biopsy showing basal layer degeneration, cytoid bodies and a chronic inflammatory cell infiltrate. (Haematoxylin & eosin, × 90).

applied to the proximal nail folds led to a slight reduction in the swelling and tenderness but no significant improvement of the nail dystrophy. Our patient has been unwilling to have intralesional steroid injections and systemic steroid therapy has not been considered because of her primary biliary cirrhosis.

Subsequently, topical Psoralen plus UVA irradiation (topical PUVA) was used. A 0·15% emulsion of 8-methoxypsoralen (Deltapsoralen, Delta Pharmaceuticals) was applied around the proximal nail folds 1 h prior to irradiation using a Waldmann hand/foot UVA unit. After treatment on 3 days a week for 6 weeks (total UVA dose 64 J/cm^2) there was a marked reduction in the degree of swelling and tenderness around the nails. Continuation of therapy with 5 J/cm^2 twice a week was necessary to prevent relapse. Liver function tests taken during topical PUVA therapy have not deteriorated.

DISCUSSION

Nail changes occur in 10% of patients with lichen planus.[1] The clinical features vary according to the part of the nail unit affected by the disease. Involvement of the nail matrix results in longitudinal ridging of the nail plate with distal notching and advanced disease leads to pterygium formation with eventual permanent nail loss. Involvement of the nail bed causes onycholysis and occasionally subungual hyperpigmentation. Although no changes are pathognomonic, pterygium formation is highly suggestive of lichen planus. In suspected cases, where confirmatory signs of skin or mucous membrane involvement are absent, biopsy of the nail may be necessary to establish the diagnosis. A longitudinal biopsy allows both the nail matrix and bed to be studied. It has been recommended that biopsies are taken from severely diseased nails to reduce cosmetic deformity.[2] An alternative is to perform a lateral–longitudinal biopsy which preserves the appearance of the nail with slight diminution of the nail width.[1] In this case the biopsy was taken from the left great toe because the nail had been lost several years previously.

There have been 23 reported cases of histologically confirmed lichen planus affecting the nails in isolation.[2–14] The features varied from mild changes, such as longitudinal ridging, to severe disease with pterygium formation and permanent nail loss.[3] Our patient is similar to these cases in having histologically proven lichen planus confined to the nails but unique in that the disease occurred in association with primary biliary cirrhosis.

The association between lichen planus and primary biliary cirrhosis was first alluded to when Seehafer reported six patients who developed the condition after receiving penicillamine.[15] Subsequently, five patients were reported who developed lichen planus in association with biliary cirrhosis—only one of these patients had received penicillamine.[16] Follow-up data from Seehafer's series revealed a total of 24 patients with lichen planus and primary biliary cirrhosis, seven of whom had never received penicillamine.[17] These data suggest that in patients with primary biliary cirrhosis, lichen planus can occur either spontaneously or in association with penicillamine therapy.

There is additional evidence to support the association of these two diseases: both are thought to be caused by abnormalities of immune function and are associated with a variety of other autoimmune disorders.[16] In patients with chronic graft-versus-host disease liver involvement shows histological features of primary biliary cirrhosis and the commonest dermatosis in these patients is similar both clinically and histologically to lichen planus.[18]

This is the first reported case of lichen planus limited to the nails occurring in a patient with primary biliary cirrhosis.

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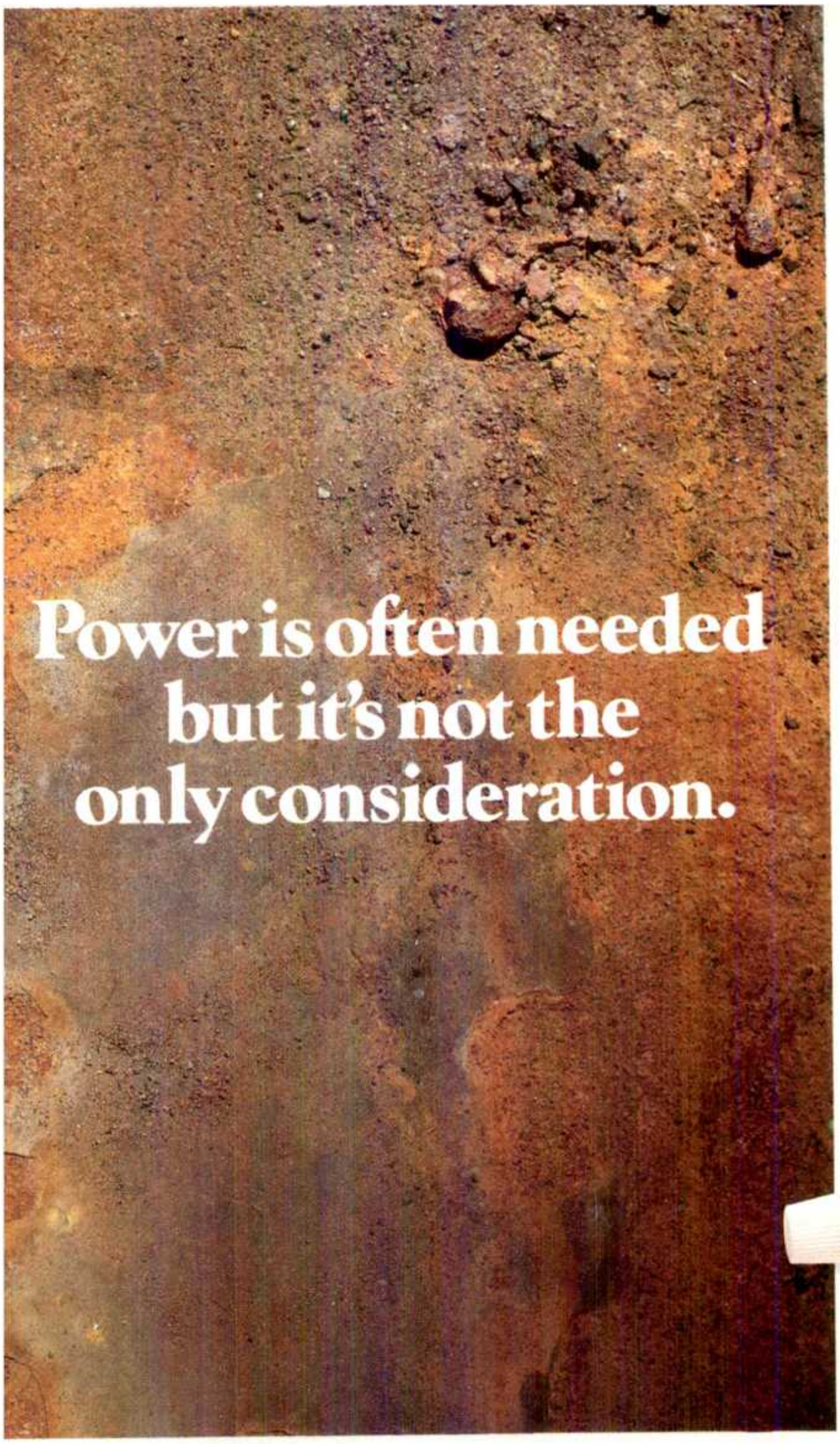

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Phospholipid esterified octadeca-9 *cis*,11 *trans*-dienoic acid in benign, premalignant and malignant skin lesions

SIR, It has been proposed that free radicals may play an important part in the pathological mechanisms involved in inflammation, ageing, and the development of cancer. Phospholipids are major components of cellular and subcellular membranes, and are clearly essential to the integrity of biological systems. Free radical attack on polyunsaturated lipids yields not only peroxides and peroxidation products but also non-peroxide diene-conjugated compounds, the measurement of which has long been established as an indicator of free radical-induced damage.[1,2] Over 95% of diene conjugation in human serum, tissue fluids and tissues is due to octadeca-9 *cis*,11 *trans*-dienoic acid (DCLA; 18:2(9, 11)), a non-peroxide diene-conjugated isomer of linoleic acid (LA; 18:2(9, 12)).[2] The molar ratio of DCLA to LA was reported to be significantly increased in exfoliated cells and biopsies from patients with cervical precancer,[3–5] although this finding has subsequently been re-evaluated.[6–8] In view of this, and since DCLA can be generated from LA by ultraviolet light (UV) irradiation (a major cause of skin cancer) in the presence of protein,[1] we sought to determine whether a relative increase in DCLA levels might be a marker for cutaneous neoplasia.

Skin samples were obtained from biopsies of a variety of benign, premalignant and malignant skin conditions (removed either by excision or by curettage) including seborrhoeic warts ($n = 16$), viral warts ($n = 17$), cellular naevi ($n = 29$), solar keratoses ($n = 7$), Bowen's disease ($n = 5$), basal cell carcinomata ($n = 19$) and squamous cell carcinomata ($n = 2$). In each case the diagnosis was confirmed histologically. Clinically normal skin ($n = 55$) was obtained from the uninvolved margin of excision biopsies (samples were taken at least 3 mm away from any lesion). Samples were trimmed of subcutaneous tissue, placed in physiological saline and stored at −20°C pending analysis. Analysis of the molar ratio of DCLA to LA in skin samples was carried out using high performance liquid chromatography as previously described.[3–5,9] The concentrations of DCLA and LA esterified as phospholipids were obtained in nmol/g of tissue wet weight for each sample, and the molar ratio was calculated on the basis of DCLA × 100/LA. The median molar ratio of DCLA to LA in the two samples of squamous cell carcinoma were respectively 2·0 and 2·79; this group did not lend itself to statistical analysis because of its small size. The results for the other conditions studied are shown in Figure 1. For the purposes of statistical analysis the group of 'precancers' was analysed together. There were highly significant increases in the DCLA : LA molar ratio for both basal cell carcinomata (BCC) and precancers as a group, compared with uninvolved control skin (C). However, an increase in the DCLA : LA molar ratio was not confined to malignant and premalignant skin conditions, since statistically significant increases in this ratio compared with control were also observed in viral warts and benign cellular naevi. The values of the DCLA : LA molar ratio in the precancers were significantly higher than those in the cellular naevi ($P = 0{\cdot}04$), but this is of questionable biological significance. There was no statistical difference in the ratio between precancers and viral warts, or between basal cell carcinomata and either viral warts or cellular naevi. The value for the DCLA : LA molar ratio in seborrhoeic warts was not significantly different from that in control uninvolved skin.

Our study has shown that, although there are statistically significant increases in the molar ratio of DCLA to LA in basal cell carcinomata and a group of premalignant skin conditions, similar increases occur in certain benign skin conditions such as viral warts and cellular naevi. Thus, an increased molar ratio of DCLA to LA is not a specific marker for premalignant and malignant tumours in human skin. This is in keeping with additional studies[6–8] which have failed to confirm the initial reports[3–5] that an increased molar ratio of DCLA to LA is characteristic of cervical carcinoma. DCLA may be generated in culture by anaerobic bacteria (notably *Corynebacterium* spp. and *Lactobacillus* spp.) isolated from the female genital tract.[7] This latter observation, together with the finding of an increased DCLA : LA molar ratio in patients with non-specific anaerobic vaginosis,[7] may explain why the initial reports[3–5] erroneously concluded that increased DCLA levels were significantly associated with cervical precancer. However, it is difficult to accept that the elevated DCLA : LA molar ratio found in the skin conditions studied here was the result of

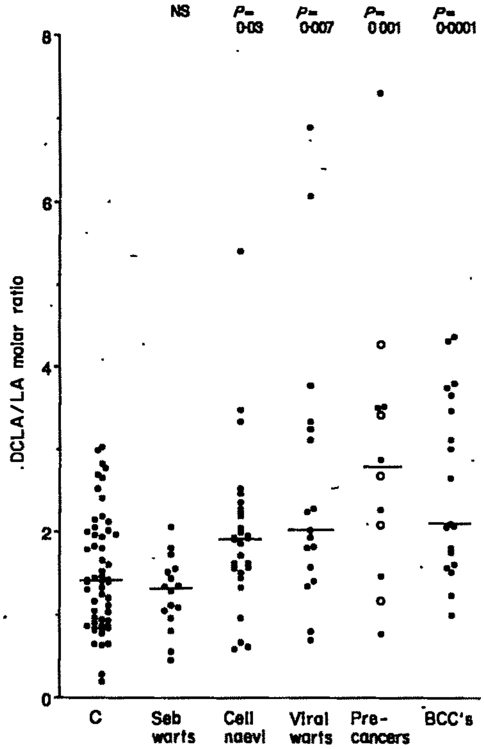

FIGURE 1. DCLA:LA molar ratio in control uninvolved skin (C), seborrhoeic warts, cellular naevi, viral warts, a group of premalignant conditions (●, solar keratoses; ○, Bowen's disease), and basal cell carcinomata (BCC). Results are expressed as medians (horizontal lines) with range. *P* values relate to statistically significant differences from control uninvolved skin (Mann–Whitney *U*-test). NS, not significant.

bacterial infection of the lesions. The increased DCLA:LA molar ratio in the various conditions is also not simply a reflection of a possible UV-induced increase in DCLA at sun-exposed sites,[1] since for the majority of lesions control skin was obtained from adjacent uninvolved sites. Thus, the biological significance of the elevated DCLA:LA molar ratio observed in the skin conditions studied remains uncertain.

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Herpetiform and mollusca contagiosa-like cutaneous cryptococcosis in a patient with AIDS

SIR, Opportunistic infections are the main feature of the acquired immunodeficiency syndrome (AIDS), and cryptococcosis occurs in 3–7% of these patients.[1] This infection is due to *Cryptococcus neoformans* and sometimes involves the skin. A wide range of cutaneous lesions are involved.[2–5] In patients with AIDS two unusual forms of skin lesions due to cryptococcosis have been reported. These are mollusca contagiosa-like lesions,[6,7] and herpetiform lesions.[8] We report a case of a HIV-positive patient who had disseminated cryptococcosis and in which both types of lesions occurred.

A 30-year-old lady from Germany was admitted to our hospital in April 1988 with a 1-month history of fever, persistent and non-productive cough and with skin and mucous membrane lesions. Three days after admission she developed headaches and had signs of meningeal irritation. The patient had a history of occasional drug abuse, and was known to have had a positive HIV ELISA test 2 years previously. On examination, there were numerous 2–5 mm herpetiform papulo-vesicular lesions on the face, some of them haemorrhagic and umbilicated. On the lower lip there was a crusted eroded herpetiform area (Fig. 1). There were many 2–10 mm dome-shaped papules that appeared like lesions of molluscum contagiosum which were present on the upper trunk and neck. There was generalized lymphadenopathy, and signs of meningeal irritation.

Tzanck smears taken from the lesions on the face, back and lip were stained with Giemsa and Indian ink and showed numerous budding encapsulated yeasts. Skin biopsies taken from two of the lesions showed intense oedema of the papillary and upper reticular dermis with granulomas containing giant cells. Abundant round bodies staining black with silver-methenamine and with clear peripheral haloes were visible (Fig. 2). These were typical of *C. neoformans*. The inflammatory response seen in these lesions was minimal.

Laboratory investigations showed an elevated ESR of 82 mm/h, haemoglobin 8·5 g/dl, platelets 123 000 and a white cell count of 3020/mm^3 with 362 lymphocytes. The subpopulations of T cells revealed an OKT4/OKT8 ratio of 0·2 (normal 1–2%). The cerebrospinal fluid (CSF) was clear with no cells and the amount of glucose was 40 mg/dl and that of protein, 35·7 mg/dl. A gram stain of the CSF fluid showed 170 yeasts per field, and both the serum and CSF cryptococcal antigen titres were 1/512. The chest X-ray showed multiple small opacities in both lower lobes, and alveolar lavage revealed numerous encapsulated yeasts. Cultures were positive for *C. neoformans* in skin, alveolar fluid and from the blood.

The patient was treated with intravenous amphotericin B (0·4 mg/kg per day) and flucytosine (150 mg/kg per day). She developed acute renal failure, and the flucytosine was discontinued though amphotericin

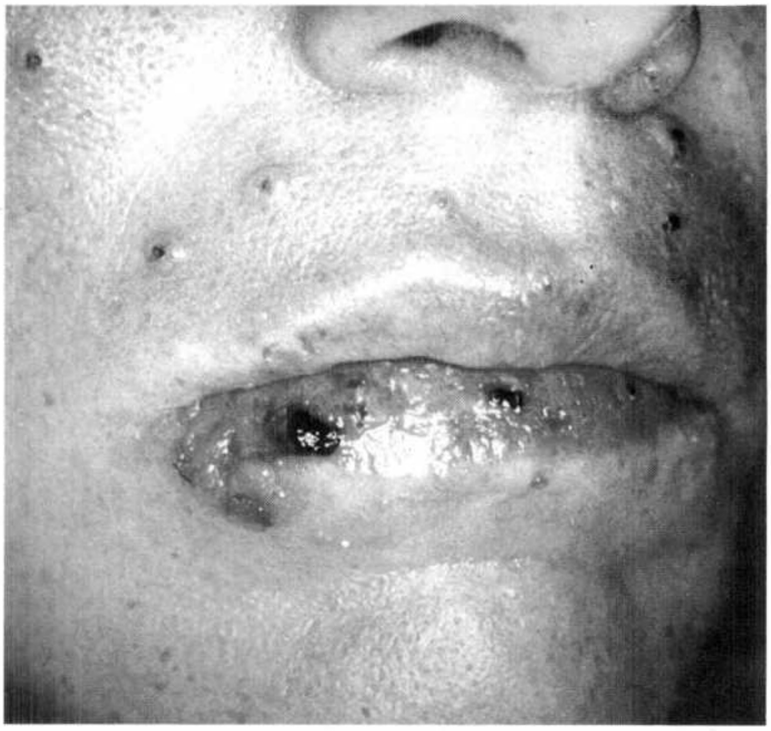

FIGURE 1. Presence of papulo-vesicular lesions on the perioral area and herpetiform-like erosion on the lower lip.

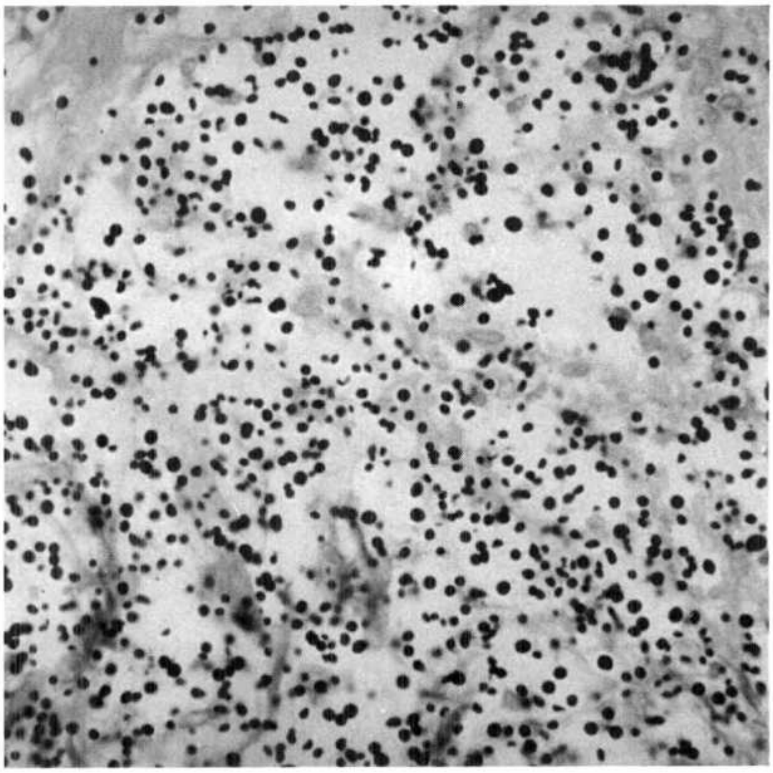

FIGURE 2. *Cryptococcosis neoformans* organisms, silver-methenamine stained.

B was maintained for a period of 2 months. There was rapid resolution of her lung and meningeal symptoms, and the skin lesions cleared in 3 weeks. The CSF and serum cryptococcal titres remained unchanged.

The presence of *C. neoformans* in the skin indicated disseminated disease,[2,3] although a primary skin infection has been described.[4] In cases of cutaneous cryptococcosis, investigations to determine other systemic diseases must be carried out. Cutaneous lesions have been reported in 10–15% of cases of disseminated cryptococcosis,[4,5] and in some they are the first sign of infection. A wide variety of skin lesions are seen.[3–5] Recently two unusual forms of skin lesions have been described in patients with AIDS.[6–8] These are herpetiform and mollusca contagiosa-like lesions. In our patient, both types of lesion occurred and were the presenting feature of this patient's cryptococcal infection.

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The effect of 'MaxEPA' in the treatment of patients with Behcet's disease

SIR, The treatment of Behcet's disease is unsatisfactory, and many of the drugs used in this chronic disease with multi-system involvement have resulted in severe side-effects.[1] Recently, dietary supplementation with eicosapentaenoic acid (EPA) has been found to be beneficial in the treatment of patients with rheumatoid arthritis,[2] atopic dermatitis and psoriasis.[3] We have treated three patients (two male, one female) with Behcet's disease with a fish oil rich in EPA. The patients all had painful oral and genital aphthae and had a positive pathergy test. All had been previously treated with various drugs that included oral colchicine, prednisolone and dapsone without any satisfactory improvement. The patients were instructed while on the trial not to have any other drugs, and received 10 capsules of MaxEPA daily that contained 1·8 g EPA. They were followed up at weekly intervals during the course of the trial that lasted 12 weeks.

During the first 4 weeks of therapy, the patients had only one episode in which they developed oral and genital aphthae, and the pathergy test became only weakly positive. After 8 weeks on the trial, two of the patients were completely free of lesions but one had a further episode of oral aphthae that resolved in a few days. After 12 weeks, none of the patients had any lesions.

Mucocutaneous lesions in Behcet's disease appear to be secondary to an immune-complex-mediated, neutrophil-related vessel damage,[4] and increased chemotaxis of neutrophils is a feature of the disease.[4] Dietary supplementation with n-3 fatty acids such as EPA decreases neutrophil chemotaxis and alters the metabolites of arachidonic acid, and results in reduction in the inflammatory response.[2] We consider that improvement in our patients on MaxEPA was not simply a placebo effect and was due to the EPA supplements. We consider that it is of use to treat patients with Behcet's disease with MaxEPA as an adjunctive therapy.

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Defective leucocyte adhesion in Papillon-Lefèvre syndrome

Sir, Recently Bergman and Friedman-Birnbaum reported on a family with a clinical variant of Papillon-Lefèvre syndrome (PLS), associated with recurrent pyogenic infections.[1] The patients had a significant improvement of their keratoderma on etretinate and a complete remission of their pyoderma. The authors suggested that etretinate may play a role in the prevention of recurrent pyogenic infections.

We studied two patients with PLS for 10 years and both presented with palmoplantar keratoderma, peridontitis and initially, severe recurrent infections (cutaneous pyodermas and liver abscesses). We demonstrated a significant decrease of chemotaxis of the patients' polymorphonuclear neutrophils (PMN).[2] Seven years later, without any treatment, these patients had not had any infectious episodes, and their palmar keratoderma had spontaneously improved. We tested their PMN functions and chemotaxis under agarose and the oxygen consumption were normal. We investigated the expressivity of adhesive protein receptors (Mac1, LFA1 and GP150 molecules) by cytofluorometry using specific monoclonal antibodies.[3] Without stimulation the result was normal but after F-Met-Leu-Phe stimulation the expression of PMN adhesion glycoproteins did not increase as it did in controls. These findings suggest an impairment of PMN adherence in PLS.

We conclude that spontaneous remission in PLS can occur without etretinate therapy and that chemotaxis and phagocytosis can improve spontaneously, but at the same time, *in vitro* PMN stimulation shows a decrease of cell adhesiveness. It would be of interest to compare PMN adhesiveness in PLS both with and without etretinate therapy.

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Neutral lipid composition of Fordyce's granules

SIR, Sebaceous glands on the vermilion border of the lip and the oral mucosa are known as Fordyce's granules or Fordyce's spots. These glands are not associated with hair follicles and have the histology of sebaceous glands with a characteristic honeycomb arrangement of sebocytes within the acini that empty their contents in a holocrine secretory process.[1] Previously, these small yellow nodules, 0·4–0·8 mm in diameter, were thought to represent degenerative changes in the epithelium of the mucosa,[2] but it is now well recognized that these glands are found in 80–90% of adults.[3]

According to the literature Fordyce's spots do not occur in the foetus, but develop postnatally.[4] The earliest age at which oral sebaceous glands have been clinically observed is 3 years,[5] although they can be histologically demonstrated in the newborn.[1,6] Puberty exerts only a slight influence on the increase in number of glands, and this is more pronounced in boys than in girls. The prevalence of the glands reaches a peak at 20–29 years of age. The number of glands per 0·5 cm^2 may range from 1 to 10 or more in a normal adult. The size of Fordyce's spots increases in males up to the age of 50–59. The density as well as the prevalence are greater in males than in females.[7]

The present study was undertaken to investigate the neutral lipid composition of Fordyce's granules, which had not previously been analysed and to determine whether there was a similarity of these lipids to the neutral lipids of cutaneous sebaceous glands.

A total of six Fordyce's granules were dissected from the lips of two adult males, and the material was immersed in 100 μl of hexane. Samples were sonicated on ice and filtered to remove bacterial and cellular debris. The lipid was analysed using thin-layer chromatography[8] and the amount of each lipid class was calculated according to the charring efficacy of known standards.[9]

The neutral lipid of Fordyce's granules was composed of sterols (5·3 ± 2·1%), sterol esters (9·4 ± 5·9%), wax esters (30·2 ± 4·8%), squalene (14·7 ± 1·4%), free fatty acids (3·2 ± 1·4%), and triglycerides (37·2 ± 5·5%) (Table 1).

TABLE 1. Neutral lipid composition of Fordyce's granules (%)

Sample	Sterol	Sterol esters	Wax esters	Squalene	Free fatty acids	Triglycerides
1	9·3	2·9	24·9	12·8	3·2	46·8
2	4·3	13·1	29·9	13·6	1·9	37·3
3	4·8	8·4	33·5	15·2	2·9	35·2
4	5·5	18·5	24·4	15·7	5·5	30·3
5	4·6	10·3	32·1	16·4	1·8	34·9
6	3·4	3·4	36·4	14·2	3·9	38·6
Mean	5·3 ± 2·1	9·4 ± 5·9	30·2 ± 4·8	14·7 ± 1·4	3·2 ± 1·4	37·2 ± 5·5

These findings demonstrate that Fordyce's granules contain neutral lipids similar to those found in sebaceous glands on the skin.

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Book Reviews

Human Hair: Volume 1. Fundamentals and methods for measurement of elemental composition; Volume 2. Trace element levels. V.VALKOVIC (1988). Boca Raton: CRC Press. ISBN 0-8493-4861-7 and 4863-5. Price £149.50.

The first four chapters provide a general review of hair growth. This is based upon earlier, more extensive reviews and does not really offer anything new for the skin biologist or clinician interested in hair. On the other hand much of the detail (e.g. on the hormonal regulation of baldness) does not seem relevant to the main subject matter of the books, i.e. analysis of trace elements in hair.

The volumes provide a detailed review of work on analysis of trace elements in human hair. They cover methods of analysis, including sample preparation and standard reference materials (eight chapters); the environmental origins of trace elements in hair are covered in detail as are changes which occur in physiological and disease states and correlations with trace element levels in other tissues. A chapter on forensic applications of hair analysis is concerned mainly with microscopic examination of hair structure. This is followed by a chapter on mathematical modelling of trace elements in hair and finally a summary of data for different elements.

There is a wealth of experimental detail provided in most chapters. However, the lack of adequate summaries makes it difficult for the general reader to sift out the most significant findings. Furthermore, there is an irritating number of minor errors in the text and tables. In summary, these volumes may be of considerable interest to those working in the field of trace element analysis, but are probably not suitable as an introduction to the subject for the more general reader with a background in dermatology or skin biology.

M.B.HODGINS

Skin Tumours—Experimental and Clinical Aspects. (Volume 11 in Carcinogenesis) Edited by C.J.CONTI, T.J.SLAGA AND A.J.P.KLEIN-SZANTO (1989). New York: Raven Press. Pp. 391. ISBN 0-88167-478-8. Price $122.50.

This book is an absolutely first-class update in the field of cutaneous carcinogenesis. I have personally read and enjoyed every chapter—not always the case in books for review.

Eighteen reviews are grouped under four broad headings: Introduction, Biology of Human Skin Tumours, Biology of Experimental Tumours, and *In Vitro* Approaches to Skin Tumour Biology. Without exception, every review is well written, and there is only one which the clinician may find heavy going—I will leave you to find this one for yourself.

A particularly welcome aspect of this book is the way in which the authors have linked the vast volume of research carried out on animal models with the human situation. Oncogenes, viral oncogenesis, chromosome alterations, and other important aspects of our current understanding of cutaneous malignancy are covered in detail and with clarity. The references are nicely balanced and are neither skimped nor overwhelming. They are pleasantly up-to-date.

Inevitably, because of the rapidly advancing knowledge in this area, the book will date. Buy it now and read it in 1989. In this way you will be ready for the developments of 1990. The book is thoroughly recommended for personal usage.

R.M.MACKIE

An Atlas of Stomatology. C.SCULLY AND S.FLINT (1989). London: Martin Dunitz. Pp. 268. ISBN 0-948269-52-9. Price: £75.

This is an attractively produced and illustrated atlas with photographs of generally high quality. The text is divided into 16 clinical sections, and the largest of these is devoted to diseases of the digestive system. The author's definition of stomatology is wide, and although I would consider any dermatological lesion on the head and neck area fair game for inclusion, I suspect that many will consider that the net has been rather widely cast when they see illustrations of the hands in dermatomyositis, the legs in erythema nodosum, and a rather poorly focused example of hepatosplenomegaly. A somewhat bizarre publisher's note at the beginning appears to warn us to wear gloves while reading the book. The message is I think meant to convey the fact that some of the illustrations show ungloved hands prior to the introduction of recommendations with regard to HIV infection. The recommendation could certainly have been better phrased.

The atlas is good value for money and is recommended for departmental library purchase.

R.M.MacKie

A Colour Atlas of Tropical Medicine and Parasitology (third edition). W.Peters and H.M.Gilles (1989). London: Wolfe Medical Publications. Pp. 240. Illustrations: 777 colour, 75 black and white. ISBN 0-7234-1534-X. Price £25.00.

The appeal of this third edition for its target readership of 'physicians, students and ancillary staff world-wide' has been enhanced considerably by its larger, clearer format and improvement in the quality of the reproduction of its illustrations. Its principal strength remains its coverage of parasitology. This subject lends itself to pictorial presentation and there are exemplary diagrams and superb photographs and photomicrographs of parasites, vectors, intermediate hosts, and habitats. The atlas also contains sufficient clinical material to be of interest to the trainee dermatologist. More than 20% of its illustrations are of cutaneous signs of tropical diseases. These are of more variable quality, but generally more than adequate.

The text is of necessity brief and concentrates on epidemiology and diagnostic features. There is no attempt to give comprehensive descriptions of course or prognosis and there is no coverage of therapeutics. The non-parasitic infections are dealt with in less detail than those due to protozoa and helminths, but there is mention of the vector-borne temperate infections such as Lyme disease. Coverage of non-infective disorders is limited to five pages on nutritional disease and a brief miscellaneous section which includes ainhum and tropical pemphigus (confusingly referred to as Brazilian foliaceus pemphigus).

At its fairly reasonable price this little book can be recommended as the most approachable and best-illustrated of the basic textbooks of diagostic tropical medicine and a useful introduction to more detailed dermatological works such as Canizares' '*Clinical Tropical Dermatology*'.

W.S.Douglas

Immunodeficiency and Skin. Edited by P.Fritsch, G.Schuler and H.Hintner (1989). Basle: Karger. Pp. 309. ISBN 3-8055-4888-5. Price US$169.50.

One of the reasons why I retained this volume for personal review was the fact that the first 10 pages of the volume were bound upside down and the wrong way round. In a book costing as much as this, this is irritating, although in the event it took only five minutes surgery to correct the problem.

The volume is the proceedings of the ESDR Meeting held in Innsbruck in February 1988 devoted to Immunodeficiency and Skin. It is divided into six main areas with a brief historian's note at the end. The main areas are the skin as an organ of host defence, clinical and laboratory diagnosis of immunodeficiency, primary and secondary immunodeficiency syndromes, skin manifestations in the immunodeficient host, AIDS, and therapy. As with all proceedings of meetings, there is a variable style, quality and value to the individual chapters. The description of the skin as an organ of host defence is in general excellent, as are the clinical and laboratory diagnoses of immunodeficiency contributions. The descriptions of the primary and secondary immunodeficiency syndromes do at times read rather like a series of rare case reports. In the field of AIDS it is interesting to see how quickly advances are coming into therapy in this area, and how relatively dated the material appears already. For example, in discussions on *Candida* there are no imidazoles mentioned other than ketaconazole. In the main section on AIDS, I personally would have welcomed a good epidemiological review tracing the development of the disease, but the other contributions are well produced.

Overall this is an interesting account of what was clearly a most enjoyable and informative meeting. It is difficult to know whether or not there are any contributions in this text which will not appear elsewhere in refereed journals. The price will deter purchase for other than departmental use.

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Books Received

Vitiligo: Neural and Immunologic Linkages. A.K.Dutla (1988). Calcutta: Indira Publications.

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News and Notices

Introductory Course on the Biology of the Skin, Cambridge, December 1989

The annual introductory course 'The Biology of the Skin' will be held in Fitzwilliam College, Cambridge from 11 to 15 December 1989. This course is primarily for registrars and postgraduate students at an early stage of their training in dermatology but is open to other interested practitioners. Closing date for applications: 24 November 1989. Further information may be obtained from the Conference Secretary (BOS), Post-graduate Medical Centre, Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K. Tel. Cambridge (0223) 212216.

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Most women with acne have polycystic ovaries

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SUMMARY

Out of 98 female referrals with acne vulgaris it was possible to define ovarian morphology by high resolution ultrasound imaging of the pelvis in 82 (84%). Sixty-eight (83%) were shown to have polycystic ovaries, compared with 19% in a control group without acne. The presence of polycystic ovaries in the acne patients did not correlate with acne severity, infertility, menstrual disturbance, hirsutes, or biochemical endocrinological abnormalities.

The polycystic ovary syndrome is an association between menstrual irregularities, obesity, features of hyperandrogenization with enlarged ovaries, a typical echo pattern on high resolution ultrasound imaging of the female pelvis and a characteristic hormonal profile.[1] Such ultrasound findings are very common in women with oligomenorrhoea or idiopathic hirsutes and have been found in 22% of a volunteer population.[2,3] Acne may be a manifestation of cutaneous hyperandrogenization in women, and has been associated with the polycystic ovary syndrome. Women with acne may have a similar profile of hormonal abnormalities.[1] We studied the prevalence of ultrasonically defined polycystic ovaries in women with acne vulgaris.

METHODS

District ethical committee approval was obtained for this study. New female patients in the dermatology clinic with a diagnosis of acne in the letter of referral were recruited. The acne, gynaecological and contraceptive histories were taken onto a standard data sheet. Patients were defined as having amenorrhoea when there had been no menstruation within the previous 6 months, oligomenorrhoea if the cycle length was greater than 35 days, frequent periods if the cycle length was less than 25 days and menorrhagia if menstrual bleeding was heavy enough to require treatment. Otherwise, patients were recorded as having a normal menstrual cycle. Fertility was recorded as either proven, with no difficulty in conceiving, untested, or as primary or secondary infertility. Patients were examined for the presence of features of cutaneous

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androgenization. Hirsutism was recorded as present if the Ferriman and Gallwey score was greater than eight, which represents the ninety-fifth centile for normal premenopausal women.[4] Acne was graded according to the method of Burke and Cunliffe.[5] Treatment for acne was prescribed in keeping with the authors' conventional practice. With informed verbal consent ultrasonic examination of the pelvis was scheduled for the mid-follicular part of the next menstrual cycle. On the same day blood was drawn for estimation of the serum concentrations of luteinizing hormone, follicle stimulating hormone, prolactin, testosterone and thyroxine.

Ultrasonography was performed by one of two radiographers (J.K. and A.P.). Polycystic ovaries were identified according to the criteria of Adams, i.e. 10 or more cysts, usually arranged peripherally, of diameter 2–8 mm, associated with an increase in ovarian stroma and volume. Borderline polycystic ovaries were defined as those of normal size but with 8–12 peripherally arranged cysts and minimal increase in stroma.[2] The ovarian diameter was measured in three planes and the mean diameter (d) and the ovarian volume calculated from the formula: ovarian volume $= 4/3\pi(d/2)^3$. Female patients attending the dermatology clinic with a diagnosis other than acne and female hospital staff who regarded themselves as healthy and had no evidence of acne were recruited as controls.

Using the SPSS/PC statistical package, the two-tailed Students' *t*-test was used for between group comparisons. Log transformed data were employed and geometric means calculated, where the data were not normally distributed. Chi-squared tests were performed (with the Yate's correction for continuity) to test the significance of differences between frequency distributions.

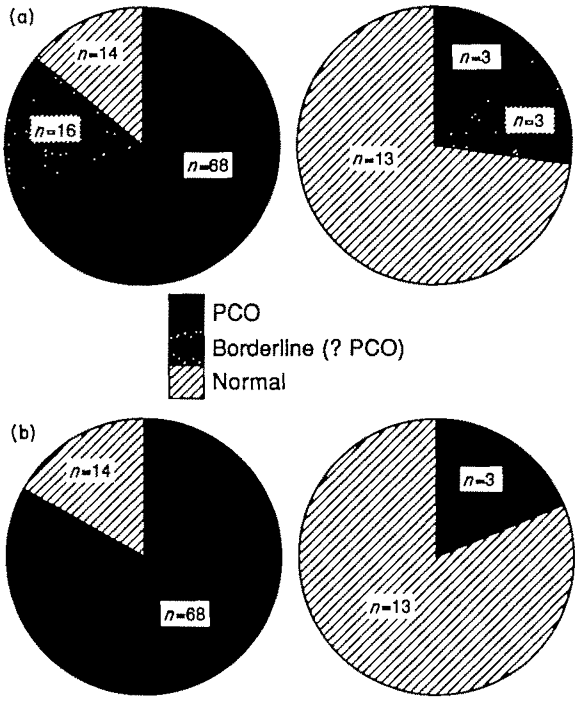

FIGURE 1. (a) Pie chart to show the prevalence of polycystic ovaries, ovaries with a borderline polycystic appearance and normal ovaries on pelvic ultrasonography in women with acne (left) compared to women without acne (right). (b) Pie chart to show the same data but excluding women whose ovaries had a borderline appearance on pelvic ultrasonography.

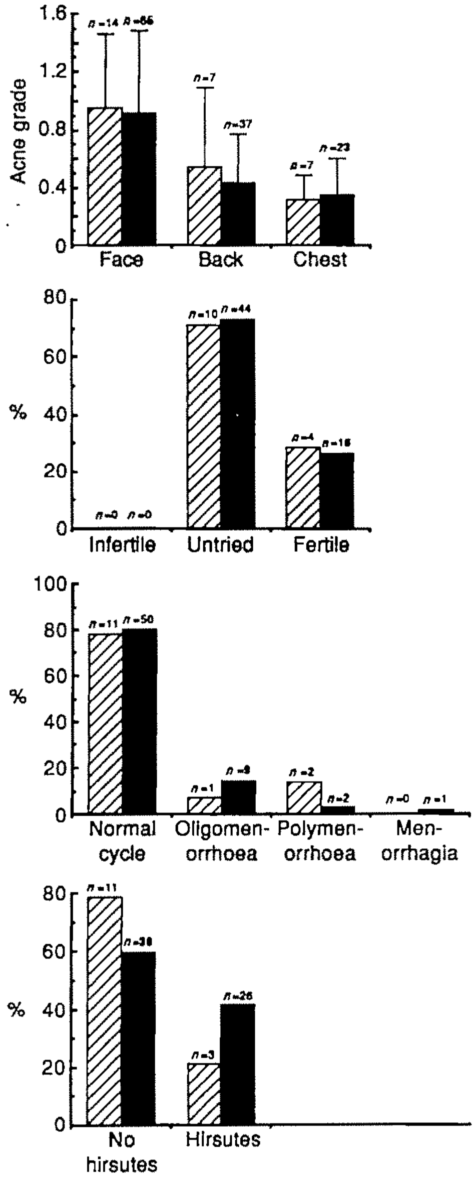

FIGURE 2. Histograms showing the frequencies of acne grade (±SD), fertility status, menstrual disturbance and hirsutes in women with acne who had polycystic ovaries on pelvic ultrasonography compared with those women with acne who had normal ovaries. ■, normal; ■, PCO.

RESULTS

One-hundred and fifty-one women with acne were recruited (mean age 25·2 years, SD 5·66), of whom 98 (65%) attended for scanning. Sixty-eight (69%) had scans diagnostic of polycystic ovaries, 16 (16%) had borderline scans and in 14 (14%) the scans were normal (Fig. 1a).

Thirty-three women were assessed for their suitability as controls (mean age 28·1 years, SD 5·8); nine were excluded because of a history and signs of acne. Of the remaining 24 (mean age

28·1 years, SD 6·34), 19 (79%) attended for ultrasound scanning. Three (16%) had polycystic ovaries on ultrasound, three (16%) were borderline and 13 (68%) were normal (Fig. 1a).

When those women with acne with borderline appearances on ultrasonography were excluded from consideration, 83% had polycystic ovaries and in 17% the ovaries were normal. Likewise, in the control group, the prevalence of polycystic ovaries was 19% (Fig. 1b). This difference is highly significant (χ^2, 24·5, $P < 0·0001$).

There was no correlation between the severity of acne and the demonstration of polycystic ovaries (Fig. 2). Approximately 70% of women in both groups were of untested fertility but similar proportions in both groups had conceived with no difficulty (Fig. 2). There was no difference in the frequency of menstrual disturbance between the two groups (Fig. 2). Three out of 14 (21%) women with acne and normal ovaries had hirsutes compared with 26 (41%) in the

TABLE 1. Arithmetic and geometric* means (with 95% confidence intervals in parentheses) of the ages, serum hormone concentrations and ovarian volumes in the different groups of women. Group 1, women without acne (controls) who did not have polycystic ovaries on ultrasonography; group 2, women with acne who did not have polycystic ovaries on ultrasonography; group 3, women with acne who had borderline ultrasonographic appearances; group 4, women with acne who did have polycystic ovaries on ultrasonography

	Group 1 No acne no PCO $n = 8$	Group 2 Acne no PCO $n = 10$	Group 3 Acne ? PCO $n = 14$	Group 4 Acne PCO $n = 61$
Mean age	32·1	26·0	24·7	26·2
(years)	(27·5–36·7)	(22·6–29·4)	(21·5–27·8)	(24·5–27·8)
n	8	10	14	58
Mean* serum	300	391	266	272
prolactin (μ/l)	(179–372)	(310–490)	(198–354)	(233–318)
n	8	10	14	51
Mean serum	95	103	101	95
thyroxine (nmol/l)	(82–108)	(82–125)	(95–107)	(89–101)
n	7	7	14	46
Mean serum	4·3	5·0	4·7	4·7
FSH (U/l)	(2·3–6·3)	(3·6–6·3)	(3·7–5·7)	(4·2–5·2)
n	8	10	14	52
Mean* serum	5·6	5·3	5·5	5·3
LH(U/l)	(4·5–6·9)	(3·3–8·3)	(3·8–7·9)	(4·4–6·4)
n	8	10	14	51
Mean serum	1·80	1·96	2·03	2·14
testosterone (nmol/l)	(1·48–2·11)	(1·50–2·42)	(1·74–2·33)	(1·96–2·32)
n	8	10	14	51
Mean* ovarian	8·4	5·6	8·8	11·8
volume (ml)	(6·4–11·0)	(4·3–7·4)	(6·6–11·5)	(9·6–14·5)
n	4	9	10	35

group with acne and polycystic ovaries (Fig. 2). This difference is not statistically significant (χ^2, 1·08, $P = 0·30$). There was no significant difference in oral contraceptive usage between women with acne who did and did not have polycystic ovaries.

Women taking the oral contraceptive pill were excluded from analyses of the biochemical endocrinological data. The age distribution of women without acne and with normal ovaries was then found to be significantly different from the ages of the women in the acne groups, making comparison of hormonal abnormalities between acne and control groups inappropriate. There was no significant difference between those women with acne whose ovaries were normal, those whose ovaries were borderline and those with polycystic ovaries in terms of the frequency distributions of their serum concentrations of luteinizing hormone, follicle stimulating hormone, prolactin, testosterone and thyroxine (Table 1). All subjects and controls had normal serum concentrations of thyroxine and follicle stimulating hormone. Seventeen (20·5%) of the women with acne, but none of the controls, had elevated serum testosterone concentrations (> 2·5 nmol/l); two (3·9%) women with acne, but none of the controls, had elevated serum prolactin concentrations (> 700 μ/l); eleven (13·3%) of the women with acne, but none of the controls, had elevated serum luteinizing hormone concentrations (> 10 IU/l). There was no significant difference in the percentages of women with elevated serum testosterone, prolactin, or luteinizing hormone concentrations between those women with acne whose ovaries were normal, borderline or polycystic.

DISCUSSION

Polycystic ovaries have been identified in 26% of women presenting to a gynaecological endocrinology clinic with amenorrhoea, in 87% presenting with oligomenorrhoea, and in over 90% with idiopathic hirsutes.[2] Polycystic ovaries were found in 22% of women who regarded themselves as normal. No mention was made of the prevalence of acne in these women although it was enquired into in the protocol.[3] We have shown that 70% of women with acne of whatever severity have polycystic ovaries on ultrasound but do not have any significant association with the other features of the polycystic ovary syndrome. These women with acne may represent a particular subpopulation of women with the polycystic ovary syndrome.

Patients with the fully developed polycystic ovary syndrome have a characteristic hormonal profile of raised plasma concentrations of luteinizing hormone and testosterone with normal concentrations of follicle stimulating hormone. Some patients have a moderate degree of hyperprolactinaemia.[1] Hyperandrogenaemia has been difficult to substantiate in acne. However, specific and sensitive assays for plasma levels of free testosterone, and other androgens, suggest that approximately 80% of women with severe acne, 50% with moderate acne and one-third with mild acne have some elevation of plasma androgen levels.[6,7] Hyperprolactinaemia has been reported in about 50% of women with persistent or late onset acne.[8] Some of the women with acne in our study had elevated serum concentrations of testosterone, luteinizing hormone or prolactin in keeping with previous findings although we have not found a significant endocrinological abnormality which correlates with the presence of polycystic ovaries as defined by pelvic ultrasonography.

The cause of polycystic ovaries and the polycystic ovary syndrome is unknown. Disturbances of adrenal androgen secretion and of hypothalamic–pituitary function have been proposed. We believe that the fundamental disturbance in the polycystic ovary syndrome involves a primary lesion in the ovary, but that the expression of the lesion involves extra-ovarian factors.[1]

The pathogenesis of acne is uncertain. An important abnormality is increased sebum production by the sebaceous gland, which is under androgenic control, and treatments which achieve a significant reduction in the sebum excretion rate consistently improve acne.[9,10] However, the role of circulating androgens in women with acne has been contentious because hyperandrogenaemia has not been consistently reported.[6,7] The finding that the local conversion of testosterone to the more androgenic 5-α-dihydrotestosterone, by the enzyme 5-α reductase, is increased in acne skin compared to normals[11] has led to the proposal that androgen metabolism within the sebaceous gland is important in the development of acne. The possible interaction of circulating steroids with end-organ factors, for example local steroid metabolism or steroid hormone receptors,[12] could explain our finding of a morphological abnormality affecting an endocrine organ distant from the skin. However, acne is probably a multifactorial disorder in which different defects (seborrhoea, ductal hypercornification, bacterial colonization) contribute to a variable degree to the final pathology. The associated abnormal cutaneous androgen metabolism may represent an extra-ovarian factor permissive for the polycystic ovary syndrome in the concept proposed by one of us.[1] These possibilities could explain our observations that a small proportion of women may have severe acne and normal ovaries, that most women with acne and polycystic ovaries do not have other features of the polycystic ovary syndrome and that some normal women without acne have polycystic ovaries.

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Skin reactivity to neuropeptides in atopic dermatitis

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SUMMARY

Adults with atopic dermatitis (AD), with respiratory atopy only and healthy non-atopic controls were given intradermal injections of substance P (SP), neurokinin A (NKA), neurotensin (NT), vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) and histamine into the normal-appearing skin on the back. The weal and flare responses were evaluated after 3, 5 and 15 min and the areas calculated using an automatic image analyser. With the three different concentrations used (1, 3 and 30 pmols) a statistically significant ($P < 0{\cdot}05$) reduction in both the weal and flare response to SP, NKA, NT and histamine and a reduced flare to CGRP was observed only in AD patients. Among those with AD there was no uniformity of response to the individual neuropeptide and in general the more severely affected showed a lower reactivity. Dose–response relationships were evaluated for SP and NT (10–320 pmols) in AD and healthy controls. In AD dose–response curves and time–course relationships were similar to controls, but at significantly reduced levels. The itch response to the neuropeptides and histamine was not different in atopics and controls. We suggest that this hyporesponsiveness in AD is the result of natural tachyphylaxis of the target structures (mast cells and blood vessels) and possibly due to a higher availability of neuropeptides in the skin or to a primary abnormal sensitivity of the blood vessels and mast cells to these peptides.

Several neuropeptides (NP) have been shown by immunohistochemistry to be present in human skin. These include substance P (SP) and neurokinin A (NKA), both belonging to the tachykinin family, neurotensin (NT), vasoactive intestinal polypeptide (VIP), atrial natriuretic peptide (ANP), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP) and others.[1–3] They have been described in primary sensory neurons (unmyelinated C fibres and small myelinated A-δ fibres) and are distributed throughout the skin, mainly around blood vessels and the eccrine sweat glands. More than one NP can be located in the same nerve fibre, e.g. SP and CGRP.[4] NPY and VIP have been shown to coexist with classical neurotransmitters:

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noradrenaline and acetylcoline respectively, in the autonomic nerve fibres of the skin.[1,5] VIP and ANP are implicated in the regulation of sweat production,[3,5] whereas SP, NKA, CGRP, VIP and NPY could contribute to the regulation of blood flow in the skin.[6,7] Experimental studies have demonstrated that at least some NP are involved in neurogenic inflammation. SP, NKA, CGRP and possibly other NP are the main mediators of the weal and flare reaction generated by axon reflexes.[8–10] There is evidence that in inflammatory disorders such as rheumatoid arthritis,[11] and in some physical urticarias,[12] NP could have a pathogenetic role.

Atopic dermatitis (AD) is an inflammatory skin disease in which the primary clinical event is an itchy erythema. Increased sweat production and scratching operate through neurogenic mechanisms which may aggravate the condition. In this study we have evaluated the skin reactivity to intradermally (i.d.) injected NP in AD patients and compared the responses with those in respiratory atopics and in healthy controls.

METHODS

Twelve patients with AD (mean age 25·6 years), five patients with respiratory atopy only (mean age 32·8 years) and 11 non-atopic controls (mean age 30·6 years) were studied. The patients with AD had active skin lesions in the typical areas involving more than 10% of the body surface. The healthy controls, without any history of atopy, were recruited from the medical staff and students of our clinic.

All the peptides and histamine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The substances were supplied as solids and stored in mM aliquots (in isotonic saline) at −80°C. Final dilutions were made in isotonic saline containing 10% (v/v) Sörensen phosphate buffer (pH 7·4) sterilized by filtration (millipore, Millex-GS 22 nm). They were shown to be hepatitis B surface antigen-free before use. Intradermal injections in a final volume of 30 μl were made in normal appearing skin of the back. The subjects had not received any drugs for at least 48 h prior to the study.

The neuropeptides SP, NKA, NT, VIP, CGRP and histamine were injected into the three groups of patients at concentrations of 1, 3 and 30 pmols, and the skin reactions were evaluated at 3, 5, and 15 min afterwards. In a second experiment the dose–response curves of only SP and NT were used in AD and in the healthy controls at concentrations of 1, 3, 10, 20, 40, 80, 160 and 320 pmols with evaluation after 3, 5, 15, 30, 45 and 60 min. A control solution of isotonic saline plus 10% Sörensen phosphate buffer was also injected.

The extent of the weal and flare reactions were estimated by tracing the contours of each reaction on transparent cellophane and the areas calculated using an automatic image analyser. The outline of the weal and flare reactions were traced and entered using a Summagraphics digitalizing tablet and TESAK (TESAK) VDC 501 graphic system interfaced to a Digital PDP 11/23 Computer. The results were corrected by subtracting the weal and flare areas occurring with i.d. injection of the control solution from those recorded for both NP and histamine. The itch response was recorded each time and graded as to severity by the patients. All i.d. injections, as well as the recording of the areas, were made by the same person.

Statistical analysis was performed using Student's t-test for paired and unpaired data. Only differences for $P \leqslant 0{\cdot}05$ were considered significant.

RESULTS

No differences in the weal and flare reactivity were observed between patients with respiratory atopy only and healthy controls. Patients with AD showed a significantly reduced flare response

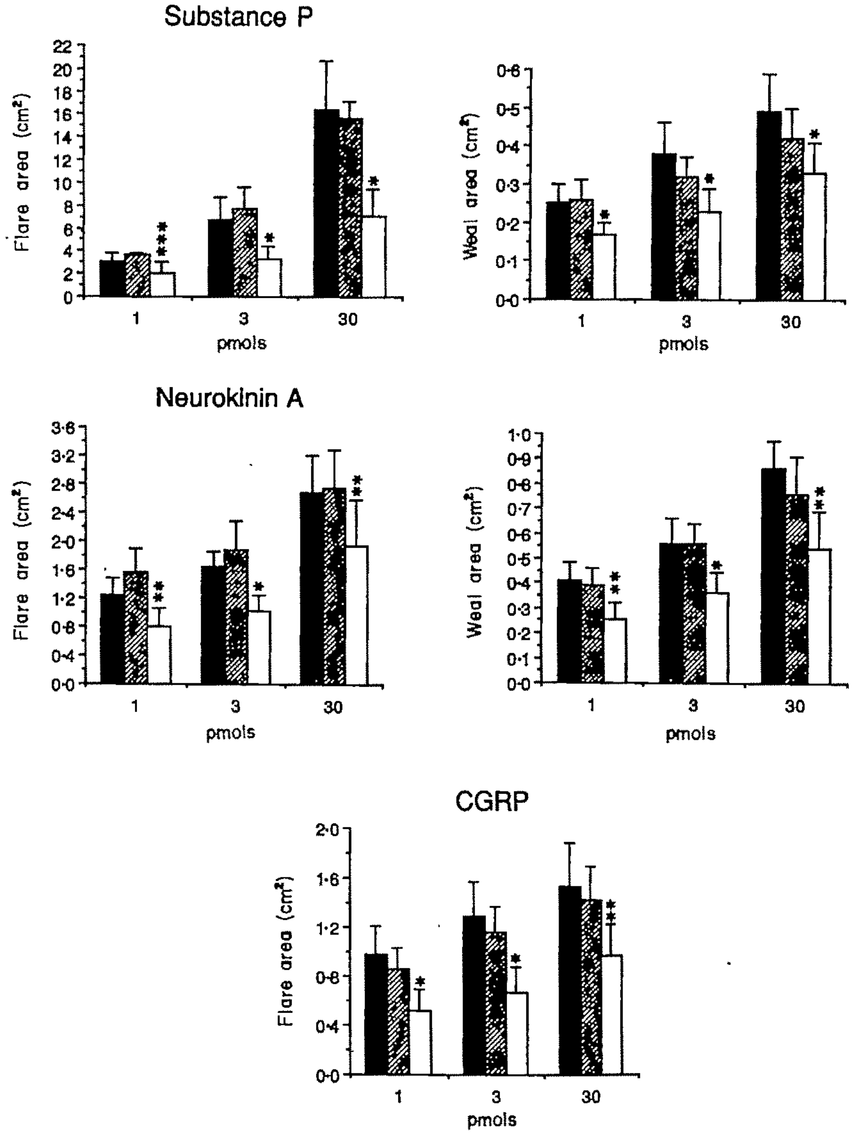

FIGURE 1.

FIGURES 1 and 2. Areas (mean ± SD) of the weal and flare response evoked by i.d. injection of SP, NKA, NT, CGRP and histamine in AD (□, $n=9$), respiratory atopics (■, $n=5$) and healthy controls (■, $n=8$). The reactions are those observed at 3 min for the flare and at 15 min for the weal, except for histamine for which both are those recorded at 15 min. The P values are those between AD and healthy controls. $^{*}P \leqslant 0{\cdot}001$, $^{**}P \leqslant 0{\cdot}005$, $^{***}P \leqslant 0{\cdot}05$.

to SP ($P = 0{\cdot}05 - < 0{\cdot}001$), NKA ($P = < 0{\cdot}005 - < 0{\cdot}001$), NT ($P = < 0{\cdot}001$), CGRP ($P = 0{\cdot}002 - < 0{\cdot}001$) and histamine ($P = < 0{\cdot}001$) and a reduced weal reaction to SP ($P = < 0{\cdot}05$ to $< 0{\cdot}001$), NKA ($P = < 0{\cdot}01 - < 0{\cdot}001$), NT ($P = \leqslant 0{\cdot}01 - \leqslant 0{\cdot}001$) and histamine ($P = < 0{\cdot}05 - < 0{\cdot}001$), with all the three concentrations used (1, 3 and 30 pmols) and at each time of evaluation (Figs. 1 and 2).

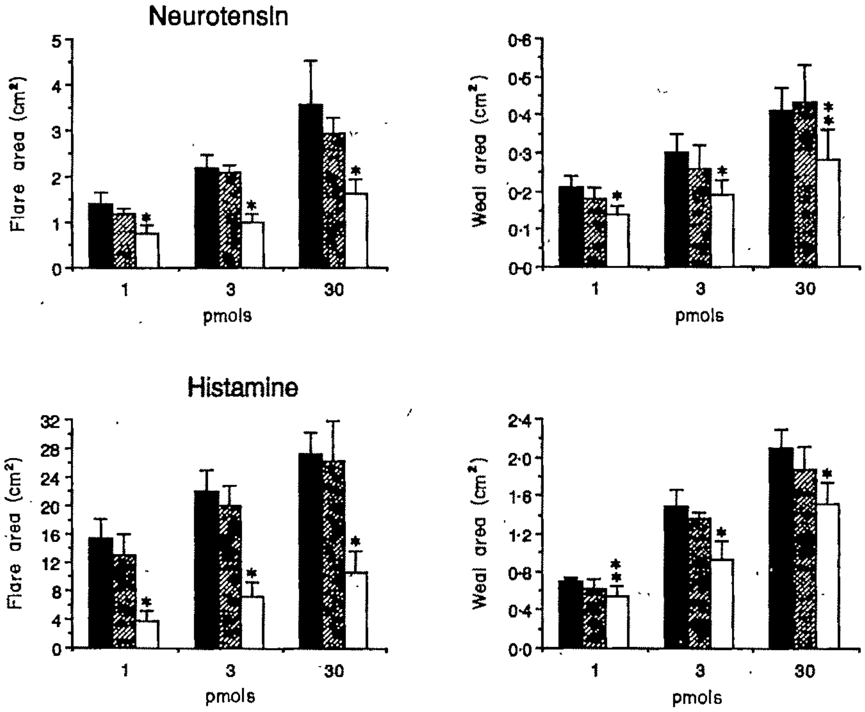

FIGURE 2.

CGRP, at the concentrations used, did not cause an appreciable oedema, but only an ill-defined hardening that was difficult to evaluate. VIP showed a slight but statistically not significant reduction in both the weal and flare reactivity (data not shown). All NP induced a greater erythema and oedema at 3 and 15 min in all the subjects. The flare provoked by histamine was greater at 15 min in the healthy controls and respiratory atopics while it was greater at 5 min in AD.

Among the AD patients there was no uniformity of response to the individual NP, and in general the more severely affected patients had a lower response. Dose-response curves for SP and NT demonstrated a dose-dependent relationship for both erythema and wealing, with a similar profile in AD and controls, but at a significant lower level in the former (Fig. 3). Time-course studies revealed that erythema reached a maximum after 3 min, whereas wealing was greatest after 15 min and then subsided slowly both in AD and in the controls (Fig. 3).

In the majority of the subjects only SP and histamine induced an itch reaction in a dose-dependent manner that lasted 5–7 min. There was no appreciable difference between atopics and controls. In a few subjects NT induced itch but only at the higher concentrations.

DISCUSSION

The best-known model for neurogenic inflammation is the axon reflex, in which the activation of nociceptors (primary sensory neurones) is followed by antidromic stimulation of collaterals, with release of NP from the peripheral nerve endings. SP, NKA and CGRP are the more accepted mediators, whereas the other peptides still have to be determined.[8–10] The i.d. injection of histamine and NP reproduce the weal and flare reaction in a dose-dependent

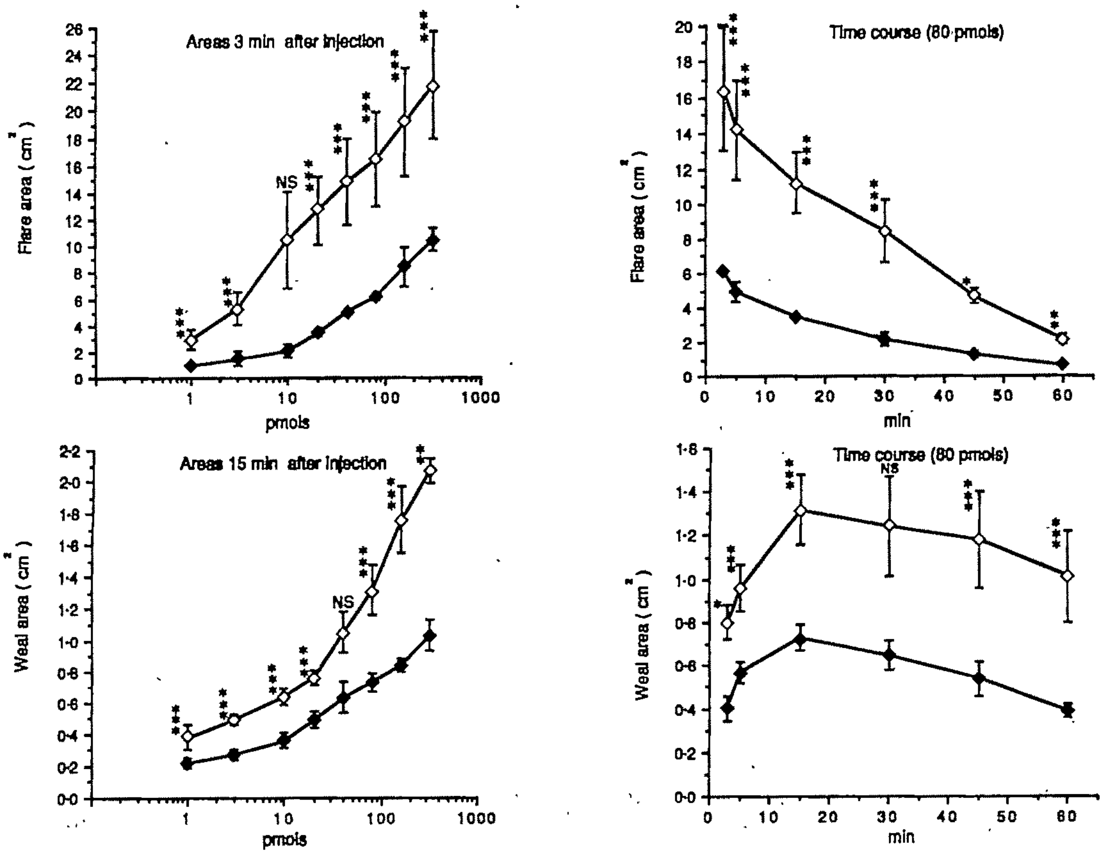

FIGURE 3. Dose-response curve (left side) and time-course relationship (right side) for the flare and the weal evoked by i.d. injection of substance P in AD (◆, $n = 3$) and healthy controls (◇, $n = 3$). The values are mean ± SD. $*P \leqslant 0{\cdot}001$, $**P < 0{\cdot}005$, $***P < 0{\cdot}05$, NS = not significant.

manner.[8-10,13-15] NP act in different ways in inducing these reactions. SP induces erythema mainly through histamine (and possibly other mediators) released from the dermal mast cells (MC), whereas the oedema is largely due to a direct action on walls of the vessels.[8-10,13,16,17] NKA and CGRP have a weak MC-releasing activity and probably act directly on the blood vessels.[8-10,14] VIP has been shown to degranulate rat peritoneal and human cutaneous MC.[13,18] NT is an histamine releasing agent, but much less potent than SP.[13,19]

In the weal and flare response observed after i.d. injection of a single NP multiple mechanisms can be involved. For example, histamine released after SP injection excites sensory nerve fibres inducing axon reflexes and with the subsequent release of other NP.[9,10]

In this study we have evaluated the weal, flare and itch response to i.d. injection of NP in the unaffected skin of AD patients. The results showed a significantly reduced weal and flare reaction to SP, NKA and NT in AD as compared to respiratory atopics and healthy controls. The erythema induced by CGRP was also reduced in AD, but it was not possible to compare the wealing because of the difficulties in recording the response.[6,10] VIP, which probably plays a major role in eccrine gland regulation, provoked a reduced (but not statistically significant) reaction in AD as compared to the control groups. The reduced weal and flare reaction of AD to histamine is already known.[20] The patients with more severe AD showed lower responses, but the degree of reduced reactivity could be roughly correlated to the severity and extension of the skin lesions. Two additional AD patients with very limited skin disease had similar reactivity to the controls. No differences in itch sensation induced by NP (SP and NT) or histamine were recorded.

The reason for the reduced weal and flare reactivity to NP and histamine in AD is not known. It may be the result of a resistance similar to the tachyphylaxis experimentally induced by repeated injections of SP or histamine.[17,21] The reduced histamine flare reaction in involved and normal skin of AD has been interpreted as due to tolerance.[16,20] This resistance of the target structures (MC and blood vessels) to exogenous NP could be due to either a higher availability of NP in the skin or to an abnormal sensitivity of the same targets in the presence of a normal release of NP, so that injected NP finds exhausted targets and evokes a reduced reaction. A close morphological relationship between nerve fibres (and peptidergic nerves) and MC has been shown in various tissues, including the skin,[22,23] and MC number is increased in AD, especially in the chronically involved areas.[24] Histamine levels have been found to be increased in both affected and uninvolved skin of AD,[25] and although normal levels were found in a later study, a higher rate of turnover can result in normal concentrations even in the case of higher histamine release from dermal MC.[26] There are no studies concerning the NP content in the skin of AD although a single case of AD was reported with increased serum levels of VIP-immunoreactivity.[27] Basophils from AD showed an enhanced releasability to immunologic and non-immunologic stimuli, such as cholinergic drugs,[28,29] while skin reactions failed to show an increased releasability to compound 48/80 or acetylcholine.[26]

Various neurovegetative skin functions have been studied in AD, especially sweat production and the vascular reactivity to autonomic nervous system drugs. Atopic patients showed a higher production of sweat throughout the year, but the results of sweat gland stimulation with cholinergic drugs are contradictory.[30,31] Abnormal vascular reactions, such as paradoxical white responses to stroking or to cholinergic drugs and nicotinic acid esters, have been interpreted as non-specific or secondary phenomena.[30,32,33]

The concentrations of noradrenaline and acetylcholine are elevated in AD.[34,35] The β-adrenergic blockade theory and the altered cyclic nucleotide metabolism are largely applicable only to peripheral blood leucocytes and have no corresponding counterparts in atopic skin.[36] However, an increased α-adrenoreceptor and/or a decreased β-adrenoreceptor reactivity has been suggested in AD.[37] Such alterations might be of importance in modifying the vascular response to NP.

Our work indicates an altered skin reactivity to injected NP in AD patients which suggests that NP might have a role in the pathophysiology of AD. The exacerbation of AD by factors such as sweating, scratching or emotional stress could involve such neurogenic mechanisms.

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Imaging the hamster flank organ by an ultrasonic technique: a new approach to animal tests

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SUMMARY

Images of the hamster flank organ were obtained *in vivo* by high resolution ultrasound (B-scan). The planimetric measurements of the ultrasonic image that determined the size of the flank organ, with a reproducibility of about 10%, highly correlated with the measurements of histological sections. This new method provides a simple and non-invasive method for determining the size of the hamster flank organ after various treatments which does not involve the killing of the animal under investigation.

The hamster flank organs (FO) are androgen-dependent paired structures on the dorsum of the animal[1] and the constituent cells respond to hormonal stimuli.[2] In the immature male and mature female, the FOs are underdeveloped and appear as slightly pigmented small spots of about 2 mm in diameter. In the mature male, the FOs are heavily pigmented areas of about 6–8 mm in diameter and are palpable due to the presence of large sebaceous glands. It is generally accepted that in both humans and animals, sebum production is related to sebaceous gland size and thus to androgenic stimulation.[3,4] The FO shows a marked similarity with the human sebaceous gland as regards its morphology, response to androgens and metabolism of hormones.[5,6] Following castration, the sebaceous glands of the FO of mature males diminish in size and resemble those of the female. The administration of androgens to immature males, castrates or mature females, results in rapid growth and increased pigmentation of the FO.[7]

The FO is, therefore, a useful animal model for determining the effect of systemically or topically applied hormones, anti-androgens and retinoids on the sebaceous gland activity.[8,9] As far as topical agents are concerned, the contralateral organ of the same animal could be used as a control, to assess a systemic activity of the drug.[10] There are several ways of assessing the activity of the FO. Invasive methods determine the FO size either by direct planimetry on

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histological sections[11] or the weight of the FO.[12] *In situ* and *in vivo* measurements of the size of the FO are required to evaluate the effects with time, and possibly the reversion at the end of treatment. In the routine *in vivo* FO test[13] the diameter of the pigmented area is supposed to correlate with the size of the FO. However, the validity of such an *in vivo* observation has been refuted by studies which found discrepancies between the diameter of the area of pigmentation and the cross-sectional area of the FO, as determined by planimetry of histological sections.[14,15] Other criteria have been used to evaluate the size of the FO, such as palpable thickening or *in vivo* gross measurements of the palpable bulk, i.e. the product of the greatest longitudinal and transverse diameters.[16,17]

Over the past few years, an ultrasonic technique has been used for the non-invasive, cross-sectional imaging of various parts of the body. By using high-frequency ultrasound transducers, resolution is improved to the extent that structures in the skin can be clearly visualized.[18–20] The aim of this study was to use the high-resolution ultrasonic imaging of the hamster FO under various physiological conditions and with different treatments, and to correlate the reliability of this non-invasive technique as regards the size of the organ.

METHODS

Chemicals

Cyproterone acetate (CA) was provided by Schering, France and spironolactone (SL) was obtained from Sigma Chemical Company. Flutamide (F) was specially synthesized by our laboratories for this purpose. Testosterone esters (Interteston®) were supplied by Intervet as a suspension, containing in 1 ml the following compounds: 6 mg testosterone propionate, 12 mg testosterone phenylpropionate, 12 mg testosterone isocaproate and 20 mg testosterone decanoate.

Animals

Syrian golden hamsters (12 weeks old) weighing 110–150 g at the start of the experiment were used. For a period of 20 days before the experiment they were kept in the light for 16 h and in the dark for 8 h each day, continuing during the experiment as this photoperiodism has been shown to influence the size of the FO.[21] Intact or castrated adult males and adult females were housed in different cages at constant temperature (25°C) and humidity (55%). Standard diet and water were given *ad libitum*. Some animals were bilaterally castrated via the scrotal route under general anaesthesia.

Treatments

Seven groups of animals (eight or nine per group, with similar body weight and FO area as determined by ultrasonic measurement) underwent the seven treatments illustrated in Table 1. These were performed over a period of 21 days and were equally distributed within each group between the left and right FO to avoid possible problems due to lateral variations.[9] Before the start of the experiment and when necessary the animals were shaved with an electric clipper.

Flutamide, cyproterone acetate and spironolactone were dissolved in acetone/propylene glycol (80/20) and 50 μl of each test solution was gently applied to one of the FOs of each animal, and 50 μl of the vehicle was applied on the contralateral organ to assess a possible systemic effect. Care was taken that the applied material did not spread to adjacent areas. Testosterone stimulation was performed through two successive intramuscular injections of Interteston® (250 μl each).

TABLE 1. Treatment groups. The topicals were randomly applied on one flank organ, daily, the contralateral flank organ receiving 50 μl of the vehicle alone

Treatment	Sex	Group (number of animals)	Administration Route	Dose
Control	Male	I(9)	Topical	
Cyproterone acetate	Male	II(8)	Topical	0·3 mg/day per FO
Flutamide	Male	III(8)	Topical	0·1 mg/day per FO
Flutamide	Male	IV(9)	Topical	3·0 mg/day per FO
Spironolactone	Male	V(9)	Topical	0·3 mg/day per FO
Intertesston® stimulation	Castrated male	VI(8)	Intramuscular injection	2 × 0·25 ml
Intertesston® stimulation	Female	VII(9)	Intramuscular injection	2 × 0·25 ml

Histology

Animals were killed by an intraperitoneal injection of pentobarbital. Rectangular strips of skin containing the flank organ were excised, processed and stained. Morphometric measurement of the area of the FO was performed using planimetry according to the method of Weissman *et al.*[16]

Ultrasonic imaging technique

The ultrasound scanner used high frequency (25 MHz), broad band focused transducers in order to optimize the axial and lateral resolutions and this has been previously described.[19,22] To create a cross-sectional image (B-scan), 80 successive A-scan lines were stored during an acquisition sequence and the image displayed on a monitor. A stepping-motor controlled by a computer moves the transducer during the acquisition time. It allows both the movement of the transducer along the skin area under investigation and the automatic relocation of the transducer. A diagram of the system used is shown in Figure 1. The apparatus gives high-resolution cross-sectional images of the skin *in vivo* with an axial resolution better than 100 μm

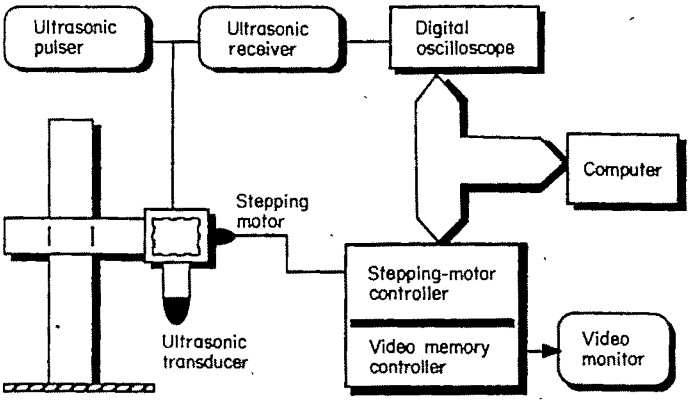

FIGURE 1. Block diagram of the high-resolution imaging system.

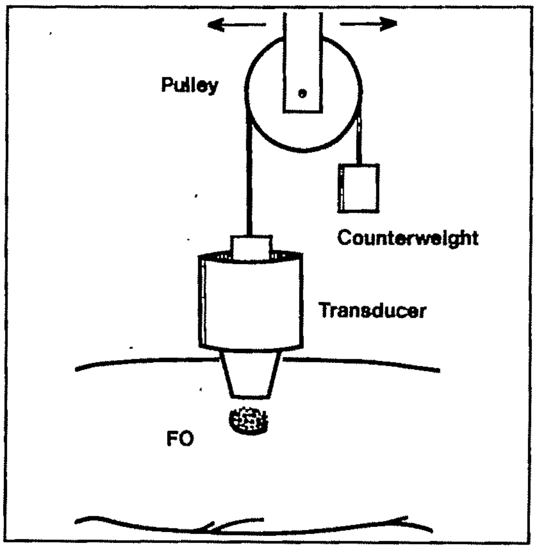

FIGURE 2. Diagram of the system used for positioning the transducer onto the flank organ.

and a lateral resolution of about 250 μm. A polaroid photograph of the image is taken for planimetric purposes.

Protocol

The animals were anaesthetized at the beginning and end of the experiment for measurement of their FOs using the high-resolution ultrasonic imaging technique. The transducer was held perpendicularly on the palpable FO to give an adequate ultrasonic image (Fig. 2). The technique was also assessed as follows:

Reproducibility. Eight successive ultrasound readings were made of the FO of two adult male hamsters, recording the greatest measured longitudinal (L) and transverse (T) diameters, depth (D) and cross-sectional area (CSA).

Accuracy. Forty-five adult hamsters, male and female, under various treatments and with various sizes of FO, were used to determine the correlation between the cross-sectional areas of FOs as determined by ultrasound and histology. One FO per animal was used for this comparison.

Practicability. The seven treatment groups were used to assess the feasibility of routine follow-up of the treatments.

Statistics

The significance of the results was tested by ANOVA followed by Tukey's test (multiple comparison of means). For each animal, the size of the treated FO was compared with both the control side and with the animals of the same group.

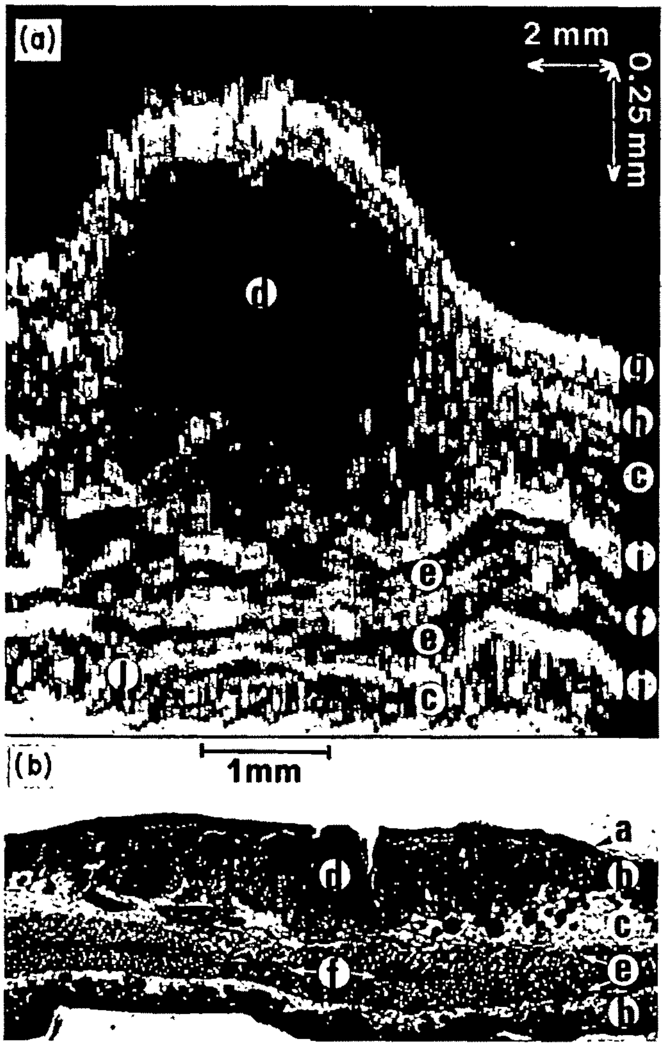

FIGURE 3. Transverse cross-sectional areas of a normal male flank organ obtained by ultrasonic imaging (a) and histology, (b) (H & E). The following structural elements can be recognized: (a) epidermis, (b) dermis, (c) hypodermis, (d) flank organ, (e) muscle, (f) cutaneous fold, (g) gel–skin interface, (h) dermis–hypodermis interface, (i) hypodermis–muscle interface, (j) skin–table interface.

RESULTS

The typical ultrasonic images of the FO in a male and a female hamster are shown in Figures 3a and 4a. The FOs shown in these figures do not have the same shape as that observed on histology (Figs. 3b and 4b), since the image obtained is necessarily compressed along the x axis. The planimetric measurements were made from the photographs.

Ultrasonic theory suggests that the 'black area' corresponding to the FO is likely to be homogeneous. Given the velocity of the ultrasonic waves, determined in the human skin[23] as 1605 ms^{-1}, the depth of the FO can be estimated at 0·6 mm (Fig. 3a). The image of the female

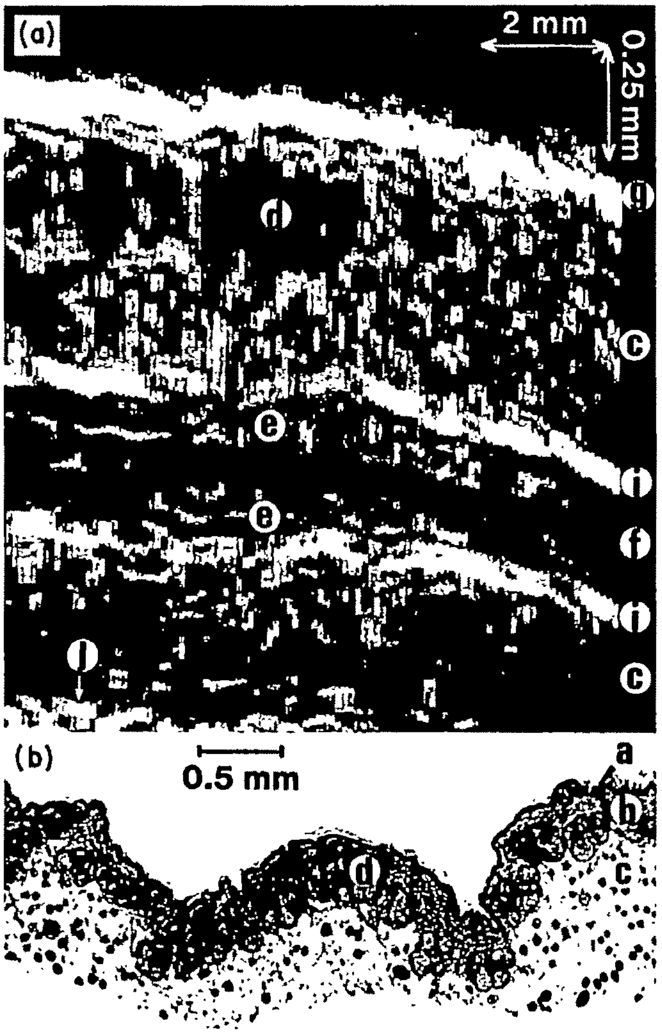

FIGURE 4. Transverse cross-sectional areas of a normal female flank organ obtained by ultrasonic imaging (a) and histology (b) (H & E). The following structural elements can be recognized: (a) epidermis, (b) dermis, (c) hypodermis, (d) flank organ, (e) muscle, (f) cutaneous fold, (g) gel–skin interface, (h) dermis–hypodermis interface, (i) hypodermis–muscle interface, (j) skin–table interface. Note that the individual smaller sebaceous glands are clearly separated from each other by fat and connective tissue.

FO (Fig. 4a) is smaller than in the male but easily distinguished, as it is in the histological sections (Fig. 4b).

Reproducibility

Table 2 shows that the measurement of T, L, D and CSA are reproducible, since they lead to low coefficients of variance (between 6 and 15%). The transverse scanning gave more consistent results (lower coefficients of variance) than the longitudinal mode, for each parameter, whereas the depth was found to be constant for both. Therefore transverse scanning was chosen for the routine assay.

TABLE 2 Accuracy and reproducibility of successive measurements of longitudinal (L), transverse (T) diameters, depth (D) and cross-sectional area (CSA) by high-resolution ultrasonic imaging. (Mean ± s.e. and coefficient of variation on two animals)

	Transverse scannings ($n = 9$)			Longitudinal scannings ($n = 9$)		
	T	D	CSA	L	D	CSA
Animal 1						
Mean	4·40	0·60	2·30	6·00	0·58	3·00
SE	0·35	0·035	0·22	0·54	0·07	0·46
CV(%)	7·95	5·85	9·55	9·90	12·1	15·4
Animal 2						
Mean	4·30	0·59	2·25	5·95	0·59	3·00
SE	0·28	0·045	0·23	0·71	0·07	0·45
CV(%)	6·50	7·45	10·2	11·9	9·85	14·8

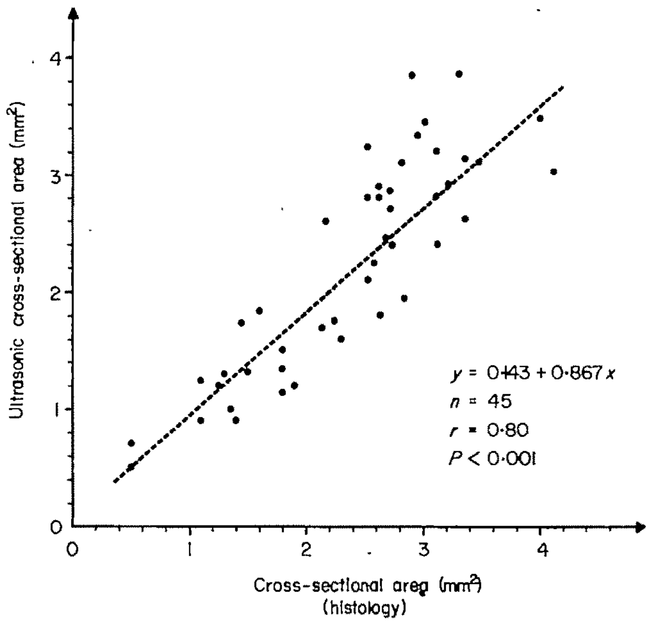

FIGURE 5. Correlation between flank organ cross-sectional areas obtained *in vitro* by histology (x-axis) and *in vivo* by ultrasonic imaging (y-axis). Both cross-sectional areas were determined by planimetry.

Accuracy

A good correlation between histology and ultrasonic image was obtained for the cross-sectional areas of the FO (Fig. 5. $r = 0{\cdot}80$, $n = 45$, $P < 0{\cdot}001$). Also, the analysis of the data in 143 control and treated animals, where both left and right FOs were measured, showed a similar size of the two organs (Fig. 6. Left vs. right: $r = 0{\cdot}94$, $P < 0{\cdot}001$).

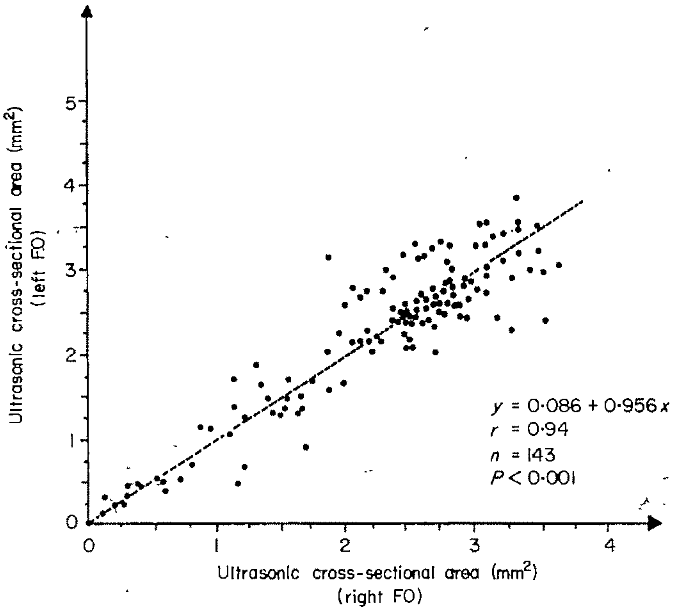

FIGURE 6. Correlation between cross-sectional areas of right and left flank organ determined *in vivo* by ultrasonic imaging after various topical and systemic treatments.

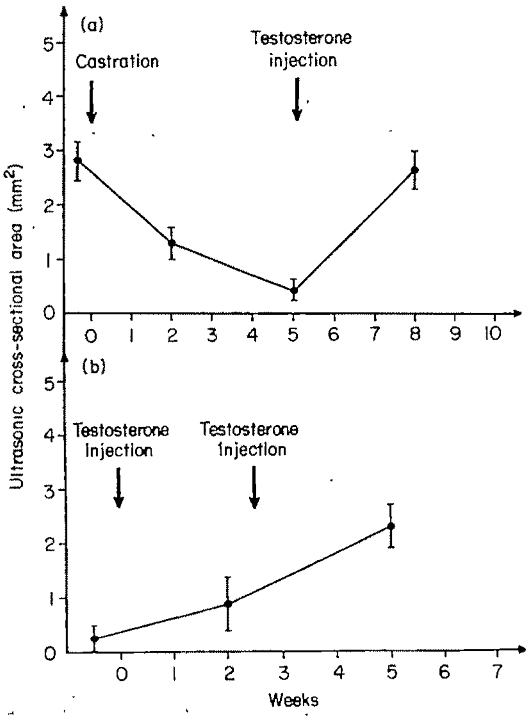

FIGURE 7. Changes with time and treatments in the cross-sectional areas (mean ± SE) of both left and right flank organs of eight male (a) and nine female (b) mature hamsters, measured by ultrasonic imaging. (a) Changes in males following castration and further testosterone supplementation. (b) Changes in females following testosterone supplementation.

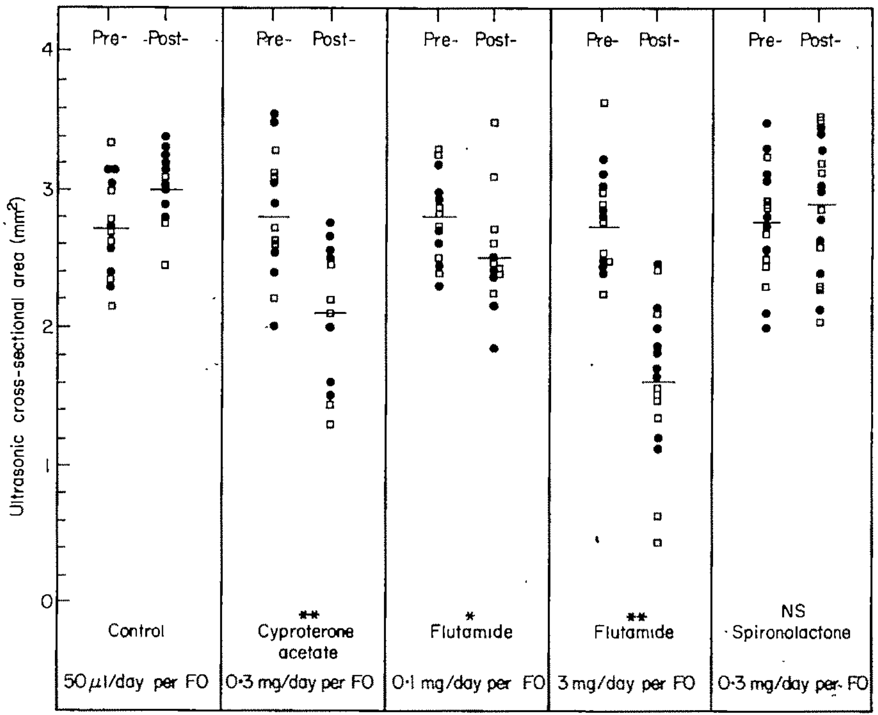

FIGURE 8. Ultrasonic cross-sectional areas of flank organs of male mature hamsters, following 21 daily topical applications of various anti-androgens. Pre- and post-treatment experimental data and means are given for each treatment. The topicals were applied on one flank organ (●), randomly chosen. The contralateral flank organ, receiving the vehicle alone was used as control (□). In this figure, all the values are pooled (treated and contralateral untreated), to illustrate the systemic effect of the applied compounds. (*), S ($P < 0.003$); (**), S ($P < 0.001$); NS, non-significant.

Castration and testosterone stimulation

Five weeks following castration, the size of the FO was reduced by about 80%. Intramuscular injections of testosterone esters reversed the effects of castration on these animals (Fig. 7a). A comparable result was obtained in female hamsters (Fig. 7b) where injections of testosterone esters led to a large increase (about 500%) in the size of the FOs over a period of 5 weeks.

Anti-androgen treatments

Topically applied cyproterone acetate (CA) (0·3 mg/day per FO) for 3 weeks led to a significant decrease (average 40%) in the size of both the CA-treated and the vehicle-treated FOs ($P < 0.001$, Fig. 8). In the group treated with 0·3 mg/day per FO of spironolactone, we found no significant changes in the treated side or the vehicle only side compared to the control group which received the vehicle alone (Fig. 8) There was a significant difference between the flutamide treated side (0·1 mg/day per FO) and the vehicle treated FO ($P < 0.003$, Fig. 8). Treatment with 3 mg/day per FO of flutamide resulted in a 40% bilateral reduction on average after 3 weeks. These changes are significant ($P < 0.001$) when compared to the pretreatment values of the same organs, and with controls (Fig. 8).

DISCUSSION

To our knowledge, these are the first *in vivo* high resolution images of male and female FOs using an ultrasonic technique. The FO is appropriate for this technique since there is a clear boundary of tissue density ('dark area') that corresponds to the large sebaceous glands that occupy the dermis. As observed in histological sections, the female FOs are much smaller than those of the males where the structures are large and homogeneous and better defined.

Our findings show that the B-scan ultrasonic technique can provide an accurate, simple and non-invasive method for measuring the size of FOs. In our experimental conditions, a coefficient of variance of about 10% on transverse repeated readings of a particular site can be considered satisfactory. Though not significantly different, coefficients of variance are slightly higher when the transducer scans longitudinally across the FO. Due to the shape of the FO, positioning the probe in the transverse axis influences the measurement less than with the longitudinal axis.

A good correlation was observed between the *in vivo* ultrasonic measurements and that of the histological cross-sectional areas of FOs. The ultrasonic technique provides an alternative to histology, without the need to kill the animals. Also each animal provides its own control and there was a highly significant correlation between the values of the right and left FOs irrespective of the treatment.

This study was aimed at demonstrating the ability of high-resolution ultrasonic imaging to discriminate between treatments, and to establish the sensitivity of the technique. Our measurements using various treatments are in agreement with previous studies obtained using different methods.[12,24,25] However, contrary to a previous study,[17] we failed to demonstrate that spironolactone decreases the size of the male FO. We suggest that this resulted from the inability of the compound to penetrate the sebaceous structures. No effect of spironolactone was found in man,[26] and the drug did not reduce the sebum excretion rate, and it was thought that topical spironolactone reduced sebaceous gland size in hamsters due to the vehicle used in the study (5% isopropyl myristate in isopropyl alcohol).

Although high-resolution ultrasonic imaging cannot provide as much information as histology it is a simple, non-invasive and accurate way of measuring the variation in the size of the FO, and can be of use for research and in the screening of new treatments, especially where histology is inappropriate.

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Systemic interleukin 1 administration suppresses arachidonic acid-induced ear oedema in the mouse

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SUMMARY

Recombinant human interleukin 1 beta (IL-1β), given intraperitoneally to mice as a single injection, significantly suppressed the development of arachidonic acid (AA)-induced ear oedema. This effect was noted 2 h after administration and for at least 5 days afterwards. IL-1β was effective in the dose range of 250 ng–20 μg/mouse. Injection of IL-1β *per se* resulted in erythema of the ears, and thus, IL-1β has the capacity not only to induce and augment but also to suppress inflammatory responses. Indomethacin administered as subcutaneously-implanted pellets did not influence the IL-1β induced-ear erythema, but suppressed to some extent the effect of IL-1β on the AA-induced ear oedema.

Interleukin 1(IL-1) is a cytokine involved in the non-specific response of the host to micro-organisms and tissue injury. The activities of IL-1, first described as functions of a macrophage product,[1] are now attributed to a family of molecules that are regulated by several genes and produced by various cells such as keratinocytes,[2] endothelial cells[3] and the glial cells of the nervous system.[4] IL-1 serves as a differentiation or maturation factor for bone marrow stem cells[5] and for cells of the immune system: thymocytes,[6] T cells,[7] B cells[8] and NK cells.[9] It also plays a role in inflammation by producing pyrexia,[10] activating endothelial cells[11] and fibroblasts[12] and in the production of acute phase proteins by the liver.[13]

Recent *in vivo* studies in the mouse and the rabbit show that local IL-1 injections produce inflammation with oedema and an accumulation of neutrophils[14] and act as a mediator for the Schwartzman reaction.[15] In other experiments systemic administration of IL-1 suppressed adoptive transfer of contact sensitivity but only when the recipient is treated not the donor,[16] suggesting that IL-1 modulates the movement of specific effector cells or the inflammatory response evoked by antigen-specific immune cells. In this study we show that systemic administration of IL-1β can down-regulate inflammation by suppressing the development of oedema in the arachidonic acid(AA)-treated mouse ear.

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METHODS

Animals

Female BALB/c mice, 8–9 weeks of age were obtained from Charles River Laboratories, Wilmington, MA, U.S.A. The mice were caged in experimental groups of five animals each and fed standard pellets and water *ad libitum*. The animals were kept for 1 week in our animal facilities before the experiments.

Chemicals

Arachidonic acid (Sigma Chemical Co., St Louis, MO, U.S.A.; grade 1, approx 99%) was dissolved in methyl-ethyl-ketone (MEK) at 50 mg/ml and stored at −20°C for no longer than 1 week. [^{125}I]-bovine serum albumin (BSA) (Du Pont NEN Research Products, Boston, MA, U.S.A.) was diluted in RPMI 1640 with 0·1% BSA at 2 μCi/ml and stored at −70°C and used within 1 month. Recombinant human interleukin-1 beta (IL-1β) was obtained from *E. coli* clone DP516 as described[17] and diluted in 0·3 M glucose before injection. The dose range used has been shown to produce dose-dependent haematopoietic stimulation in mice.[18] Control animals were injected with vehicle alone. As an additional control IL-1β was heat-inactivated at 90°C for 30 min and given in a dose of 20 μg/mouse. Indomethacin (0·5 mg) or placebo pellets (Innovative Research of America, Toledo, OH, U.S.A.) were implanted subcutaneously at least 2 days before the start of the experiments. The indomethacin dose delivered by these pellets was the highest that could be tolerated.

Evaluation of AA-induced ear oedema

This experimental model has been described in detail elsewhere.[19] The mice were injected with 1 μCi of [^{125}I]-BSA in 0·5 ml of RPMI 1640/0·1% BSA in a tail vein. One hour later 10 μl of AA in MEK was applied to each side of the right ear while the mouse was under ether anesthesia. The left ear remained untreated as a control. After another hour the animals were killed by cervical dislocation and 6-mm diameter discs of tissue were obtained from the tips of the ears by means of a disposable skin biopsy punch. The radioactivity in the tissue discs was measured in a LKB CompuGamma Counter. The radioactivity recovered from the control ear was considered to give an estimate of the capacity of the vasculature, while the difference between the radioactivity in the AA-treated right ear and the left control ear reflected the AA-induced oedema due to vasodilation and increased vascular permeability.

Statistics

The differences between group means were assessed by Student's *t*-test.

RESULTS

Time course of IL-1β effects

Application of 1 mg AA to the mouse ear produced both erythema with visible vasodilatation within minutes and oedema that was most marked at 1 h. When 20 μg of IL-1β was given intraperitoneally before the application of AA, it was found that the extravasation of [^{125}I]-BSA was significantly suppressed. This effect was present within 2 h of administration and was observed over the following 5 days (Fig. 1).

IL-1β administration by itself also resulted in increased tissue radioactivity in the untreated

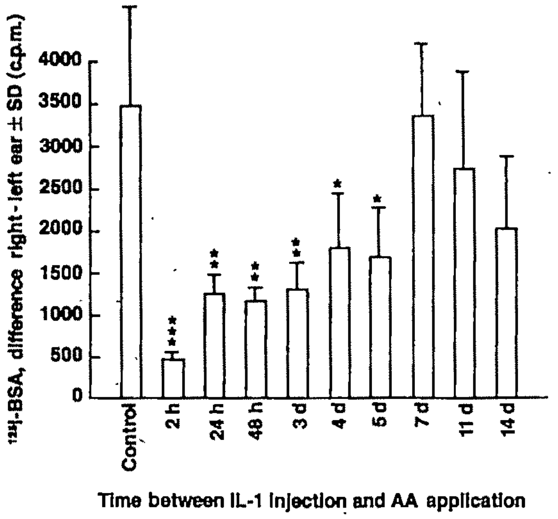

FIGURE 1. Time course of IL-1β mediated suppression of the AA-induced ear oedema. Before topical application of AA, groups of mice ($n = 5$) were injected intraperitoneally with 20 μg IL-1β at time points indicated. The ear oedema was evaluated as the difference in radioactivity (^{125}I-BSA) between the AA-treated and the untreated control ear. ***, $P < 0{\cdot}001$; **, $P < 0{\cdot}01$; *, $P < 0{\cdot}05$ indicate significance levels of differences between control and IL-1β treated groups.

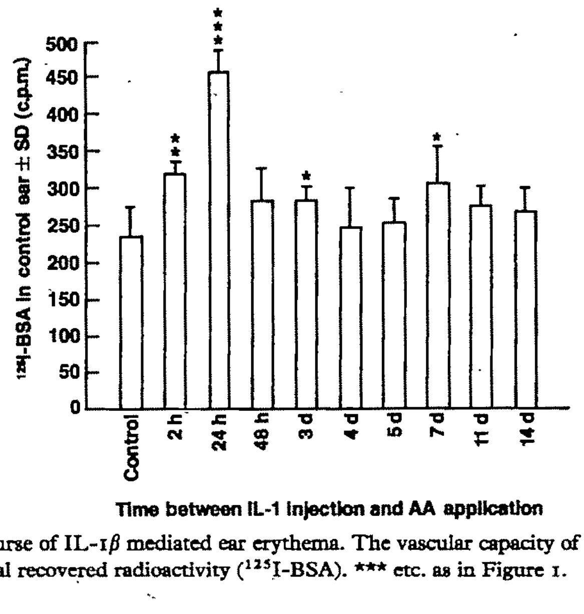

FIGURE 2. Time course of IL-1β mediated ear erythema. The vascular capacity of the control ears was estimated as the total recovered radioactivity (^{125}I-BSA). *** etc. as in Figure 1.

control ears with a maximum at 24 h (Fig. 2), but this effect was less marked than the effects of AA. This is in accord with the visual appearance of the ears in that the IL-1β treated mice had erythematous ears from 6–8 h and up to 48 h after IL-1β injection. We also found that indomethacin treatment did not modulate the appearance of erythema on the control ear.

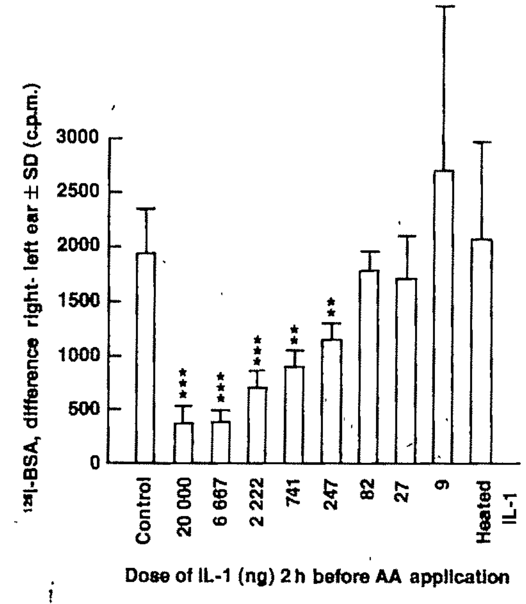

FIGURE 3. Dose response of the IL-1β mediated suppression of the AA-induced ear oedema. Serial dilutions (1:3) of IL-1β were injected intraperitoneally *** etc. as in Figure 1.

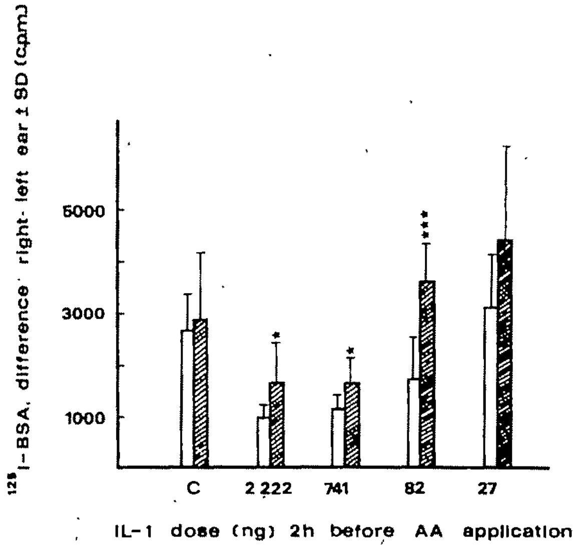

FIGURE 4. Indomethacin effects on the dose response of IL-1β mediated suppression of AA-induced ear oedema. C, control animals. ▨, indomethacin treated mice. *** etc. as in Figure 1, giving the significance levels of comparisons between animal groups ($n = 5$) with and without indomethacin treatment.

Dose response of IL-1β effects

Within the dose range from 6 μg to 82 ng/animal there was a good relationship between IL-1β dose and the suppression of the oedema, when IL-1β was injected 2 h before AA application (Fig. 3). Injection of heat-inactivated IL-1β did not demonstrate suppression of the oedema. Indomethacin treatment abrogated the IL-1β-induced suppression at lower doses and showed some modulation of the IL-1β effect at higher dose levels (Fig. 4).

DISCUSSION

Topical application of AA to the mouse ear results in erythema and oedema, preceded by the appearance of PGE_2 and LTC_4/D_4 in the tissues.[20] Experiments including topical inhibitors of the cyclooxygenase and the 5-lipoxygenase pathways suggest that the erythema (vasodilation) is due to PGE_2, while the increased vascular permeability causing oedema is mainly due to LTC_4/D_4.[21] However, other pharmacological agents such as histamine antagonists, phosphodiesterase inhibitors and free radical scavengers can also reduce the oedema suggesting that the pathophysiology of the AA-induced ear oedema is complex.[22] The endothelial cells are considered to be important in both the vasodilation[23] and the development of oedema.[24]

Our experiments have demonstrated that systemic administration of recombinant human IL-1β in the mouse was associated with a marked suppression of the AA-induced ear oedema, and this effect lasted for 5 days following IL-1β administration. The IL-1β pretreatment must have rendered the endothelial cells less susceptible to the various vasoactive mediators operating after topical AA application. The effect of IL-1β on the endothelial cells can be either direct or indirect through the generation of other cytokines or vasoactive agents. IL-1 has been demonstrated to induce production of platelet activating factor,[25] surface adhesion molecules[26] and procoagulant activity.[27] The net result of both direct and indirect IL-1β effects can be observed in the untreated ears, serving as controls in our study.

Recently it has been demonstrated that IL-1 injections into mice produce a rapid increase of serum levels of ACTH and corticosterone with maximum levels achieved 2 h after IL-1 injection.[28] The glucocorticoids not only suppress IL-1 production[29] but also some of the biological activities of IL-1.[30] One mechanism behind the anti-inflammatory effects of the glucocorticoids is the induction of regulatory proteins, such as lipocortins inhibiting phospholipase A_2 and thereby both cyclooxygenase and lipoxygenase pathways.[31] However, in our experiments AA was supplied in excess from external sources and therefore the contribution by endogenous AA would be insignificant and the role of lipocortins negligible.

The protracted effect of a single IL-1β injection over 5 days could be due to slow exchange kinetics between IL-1β and its receptor and/or to a slow internalization of the IL-1/IL-1 receptor complex.[32] In addition, the IL-1β dose used in these experiments has been demonstrated to produce prolonged circulating levels of IL-1[33]. Doses of 10 μg IL-1β/mouse have had effects on the haematopoietic system up to 4 days after administration.[18] Another reason for a sustained response to IL-1 may be glucocorticoid induction of high affinity IL-1 receptors over a period of time.[34] Our results, showing a long-lasting effect of IL-1β, are in line with previous *in vivo* studies showing an IL-1 inhibition of the ability of mice to elicit a contact sensitivity response after adoptive transfer of primed lymph node cells.[16]

Systemic indomethacin administration with the implantation of slow-release pellets did not influence the IL-1β induced erythema of the control ears. This may suggest that IL-1β induced erythema is not mediated through prostanoids generated via the cyclooxygenase pathway. Also indomethacin did not inhibit the AA-induced ear oedema, and this agrees with previous studies that have reported AA ear oedema inhibited by topical but not by systemic indomethacin.[35] However, indomethacin suppressed the IL-1β modulation of the AA-induced ear oedema to some extent. The finding that IL-1 also modulated contact sensitivity through an indomethacin sensitive mechanism[16] could be explained by IL-1 effects on the endothelial cells rather than the lymphoid cells alone.

IL-1 is usually described as a hormone involved in the propagation of inflammation, while various tissue-specific(?) inhibitors modulate its activity.[30] The present results demonstrate that IL-1β, at a high concentration, can also suppress the vascular phase of the inflammatory response in the mouse.

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Potential for irritation increases from the wrist to the cubital fossa

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SUMMARY

The effect of site on the irritant dermatitis potential was studied in 10 subjects using the volar surface of the forearm. Erythema scoring and transepidermal water loss (TEWL) measurements were used to assess irritation at different levels on the forearm. A sodium lauryl sulphate (SLS 2 g/v%) solution was used as a standard irritant in a patch test procedure and on open tests. Both the erythema scores and measurements of the TEWL of exposed skin showed significant localization differences, and demonstrated that the same sites on the forearm should be used for irritancy tests and possibly physiological studies.

In assessing the irritancy of topical agents in humans, subject and regional differences in the sensitivity of normal skin must be considered.[1–4] Intraregional differences in skin sensitivity have been noted,[5,6] and the skin on the volar forearm has been used to study the irritancy of topical agents using visual scoring systems and instrumental techniques.[6–10] We have studied the differences in sensitivity of the volar surface of the forearm to sodium lauryl sulphate (SLS) by open tests and on patch testing. We report that the potential for irritation increases from the wrist to the cubital fossa.

METHODS

Skin irritation was assessed by visual scoring of erythema and by transepidermal water loss (TEWL) after application of sodium sulphate (SLS) to the volar side of the forearm. Testing was performed in a group of 10 volunteers (four males, six females; aged 22–64 years) who gave informed consent. The open testing and the patch tests were done in the same group with the open testing being carried out several weeks later and avoiding the previously exposed arm. Polypropylene chambers (Hill Top®) were used for the patch testing onto which 200 μl of a 2 g/v SLS solution in distilled water was pipetted. The chambers were applied on the skin next to the cubital fossa (distally) and next to the wrist fold (proximally), and another three chambers were applied in between at equal distances. The skin was exposed for 24 h and the TEWL was

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measured at least 15 min after removal and the visual scoring was done 30 min after removal. Delayed reactions were included in the results. TEWL measurements were also performed at comparable unexposed (control) skin sites of the other arm.

The open testing was performed with open plexiglass rings with an internal diameter of 11 mm (total exposed skin area 0·95 cm^2). Before the exposure the skin was tape stripped 15 times with gentle pressure. The rings were glued to the skin by silicone gel (Tecsil®) and applied on the skin next to the cubital fossa (distally) and next to the wrist fold (proximally), with one ring placed in between. Pipetted into the rings was 100 μl SLS 7·5 g/v in distilled water. The skin was exposed to this solution 45 min daily for 4 consecutive days. After the exposure the arm was dried with paper tissue. On the fifth day the reactions were read and TEWL measured.

In both experiments TEWL was measured with the Servomed Ep1 evaporimeter (Servomed®, Stockholm, Sweden)[11] under neutral environmental circumstances (room temperature 20–22°C and 17°C and relative humidity 59%–67% and 65% respectively.)

The skin reactions were read visually for erythema: 1+, slight redness; 2+, moderate redness; 3+, intense redness; 4+, fiery redness.[12] The skin temperature of the exposed and unexposed (control) sites was recorded using a Yellow Springs thermistor (Yellow Springs Instrument, Yellow Springs).

The variation in site temperature and the variation in site irritation as assessed by TEWL was studied for significance by an analysis of variance (repeated measures). Differences in mean erythema scores were tested by Friedman's analysis of variance by ranks (level of significance: 5%).[13]

RESULTS

The results are shown in Table 1. Both in the patch test procedure and the open test procedure the skin of the volar forearm showed a significant increase in sensitivity, as assessed by erythema scoring, from the wrist to the elbow. This increase in sensitivity was also reflected in a significant increase of the mean TEWL scores in the patch-tested skin. In the open tests the mean erythema scores also showed a significant variation, the skin next to the cubital fossa having the highest mean score. The open tests showed a slightly different TEWL pattern, the middle of the

TABLE 1. Mean erythema scores and mean TEWL scores* ($n = 10$) of sodium lauryl sulphate (SLS) patch-tested and open-tested skin sites at different locations on the volar side of the forearm. The variation between the different locations as assessed by TEWL and erythema scores is significant for exposed skin sites (patch testing and open testing). The variation between the different locations in the mean TEWL scores of unexposed skin sites is also significant ($P < 0·05$)

	Patch testing		Open testing		Unexposed skin
	TEWL ± SD	Erythema score	TEWL ± SD	Erythema score	TEWL ± SD
Elbow	24·6 ± 15·6	1·8	14·0 ± 8·1	2·0	2·8 ± 0·7
	16·5 ± 8.4	1·2			2·3 ± 0·8
	16·2 ± 7·8	1·0	6·2 ± 3·4	0·4	2·8 ± 1·0
↓	14·9 ± 8·5	0·9			4·0 ± 1·9
Wrist	14·0 ± 6·0	0·5	6·5 ± 3·0	0·1	5·4 ± 3·3

* Mean erythema score: sum of the erythema scores/*n*.
Mean TEWL score: sum of the TEWL scores/*n*.

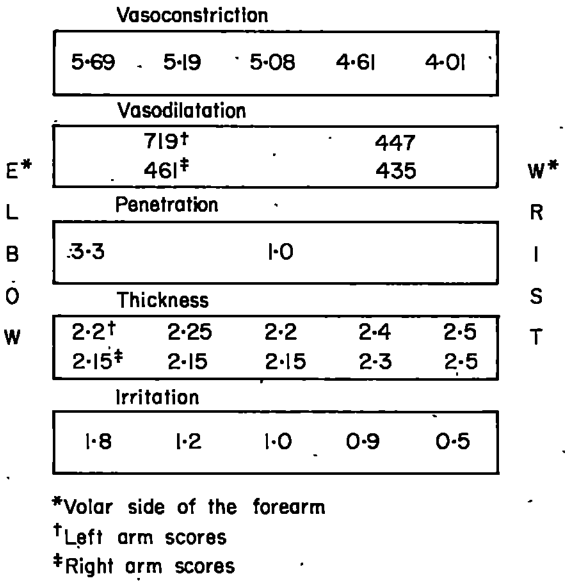

FIGURE 1. Data relevant to irritation. Vasoconstriction: mean scores in the corticosteroid vasoconstrictor assay.[14] Vasodilatation: area under the curve in mm/min after topical application of methyl nicotinate.[15] Penetration: ratio of urinary secretion after topical application of ^{14}C parathion (forearm = 1).[16] Thickness: mean skin fold thickness of the ulnar side in mm using Harpenden calipers.[6] Irritation: mean erythema score after topical application of sodium lauryl sulphate (this study).

forearm showing the lowest mean TEWL score. The mean TEWL scores of the unexposed control sites showed a gradual decrease from the wrist to the elbow. Next to the cubital fossa an increase was observed.

Mean temperature of exposed skin sites varied from 30·3°C ± 1·4 to 31·3°C ± 1·0 and of unexposed skin from 30·8°C ± 0·9 to 31·4°C ± 0·7. This variation was not significant for both unexposed and exposed skin sites.

DISCUSSION

The skin of the volar forearm proved to have an increased sensitivity to SLS from the wrist to the elbow. In open tests with SLS the skin next to the cubital fossa proved to be most sensitive. These differences may be attributable to differences in skin thickness and skin penetration and factors relating to the skin microvasculature (Fig. 1).[6,14–16] Differences in skin temperature[16] probably play an insigificant role. In our study the differences in skin temperature of the volar forearm from proximally to distally were small and not statistically significant. Also differences in contact pressure are probably not important because open testing also showed a greater sensitivity at the skin next to the cubital fossa.[17]

A good correlation between the TEWL increase and the visible response to surfactants has been reported.[8] This study showed a significant variation in mean TEWL scores of unexposed skin from the wrist to the elbow, which may have interfered with the TEWL scores of skin exposed to low molarity solutions. In this study the mean erythema score of the mid forearm was higher than the skin next to the wrist, but the mid forearm had a lower mean TEWL score in the open test compared to the skin next to the wrist. We conclude that TEWL is overestimated in this region and this may be caused by sweat gland activity,[18] in spite of the precautions to avoid thermal and emotional sweating. This overestimate negatively influences the correlation between the TEWL scores and the erythema scores in the wrist area.

From our studies we suggest that the skin next to the cubital fossa and the skin next to the wrist be avoided in quantitative and comparative investigations, because these sites are not comparable with the skin in between as regards skin irritability and TEWL. In addition, if the volar skin in between is used for studies on irritants, or if small test panels are used, rotating patterns or symmetrical sites should be used for both visual scoring and instrumental methods like TEWL to avoid regional differences.[19]

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Cutaneous fibrinolytic potential, tPA dependent, is reduced in Behçet's disease

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SUMMARY

We report our studies on the cutaneous and plasma fibrinolytic activities (FA) in nine patients with Behçet's disease (BD) as compared with nine normal controls. The euglobulin lysis time of the plasma and cutaneous fibrinolytic activity were determined in these patients. The studies showed that the plasma fibrinolytic activity was reduced in the patients with BD and there was impaired cutaneous fibrinolytic potential, tPA dependent, in those patients with venous and arterial thromboses.

Behçet's disease is a chronic and often progressive disease that is characterized by recurrent ulceration of the mouth and genitalia and is associated with iritis and, in 25% of the cases, with thrombophlebitis.[1–3] The underlying pathology appears to be that of a vasculitis[4] and a thrombotic tendency is a common feature,[5–9] with vascular thromboses, migratory superficial thrombophlebitis and retinal and vitreous haemorrhages. There may be peripheral gangrene and arterial aneurysms[4] and in addition pulmonary embolism with haemophtyses.[3,10] Fibrinolytic abnormalities[11–18] and prolongation of the euglobulin lysis time[19] with decreased levels of circulating Factor XII have been reported.[15,20] A reduced plasma fibrinolytic activity (FA) and lowered serum complement and level of Factor VIII have been reported[20] with recurrences of the disease.[15,20]

The fibrinolytic activity follows the activation of plasminogen to the proteinase plasmin that plays a key role in dissolving thrombi in the vascular tree. FA normally occurs after the release of plasminogen activators (PAs) and these can be tissue type plasminogen activator (tPA) with a molecular weight of 74000 Da, and urokinase (UK) with a molecular weight of 55000 Da derived mainly from endothelial cells. The cutaneous fibrinolytic potential is the storage in endothelial cells of PAs that are released in the skin by various stimuli such as the injection of histamine.[21,22] We report our studies on the cutaneous and plasma fibrinolytic activities in nine patients with BD as compared with similar controls.

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TABLE 1. Major clinical symptoms in nine patients with Behçet's disease

Patient	DD (years)*	Oral ulcers	Genital ulcers	Thrombosis	Acneiform skin eruptions	Arthritis	Ocular signs
1	10	+	+	+	+	+	+
2	12	+	+	+	+	−	+
3	15	+	+	+	+	−	−
4	7	+	+	−	+	−	−
5	3	+	+	−	+	+	+
6	2	+	+	−	+	+	−
7	3·5	+	+	−	+	+	−
8	6	+	+	+	+	−	+
9	1	+	+	+	+	−	+

* DD, Duration of disease.

METHODS

Nine patients with BD aged 20–62 years were studied. The diagnosis of BD was made according to the criteria of Mason and Barnes[23] and the clinical features are summarized in Table 1. All had oral lesions and treatment (levamisole, colchicine and systemic steroids) had been discontinued 2 weeks before they were studied. The control group consisted of five skin patients who had either rosacea or basal cell carcinoma and four volunteers, being matched for the sex and ages of the patients with BD. The plasma FA both in patients and controls was assessed using the euglobulin lysis time (ELT).[24]

Punch biopsies were taken from patients and controls 2 h after an injection of 0·1 ml of 0·1% histamine. The cutaneous FA in these biopsies was studied by using Todd's histochemical fibrin-slide technique.[25–27] Fresh specimens were embedded in OCT and were snap frozen. Six micron sections were cut in a cryostat and placed on cover slips that were covered with a plasminogen-rich fibrin film, obtained by mixing 50 ml of fibrinogen with 5 ml PBS at pH 7·4 with 50 μl of a thrombin solution that contained 25 U/ml and with 500 μl of PBS at pH 7·4. A control test in all cases was performed by heating the plasminogen–fibrinogen complex for 30 min at 80°C to inactivate the plasminogen, or by using a plasminogen-free fibrinogen. This assay detects about 10 mol of urokinase (UK) and is particularly sensitive to tissue type plasminogen activator (tPA). The slides were incubated in a moist chamber at 37°C for 4 h, and then stained with Harris haematoxylin. The sites of fibrinolytic activity appeared as clear punched areas of lysis in the opaque fibrin film.

The degree of FA was estimated by comparing the lysis areas with those of the normal skin and the results tabulated as to the size and number of the fibrin lysis areas around the vessels in the dermis: 0, no cutaneous FA; +, 50% less FA than found in healthy controls; + +, FA equal to that of the control group; + + +, FA more than 50% higher than the control group. This scale was considered to be valid since the tissue and fibrin film thicknesses were uniform in all the tests that were carried out.

To distinguish between UK and tPA dependent FA we used specific monoclonal antibodies (Monozyme, Lingby, Denmark) to UK and tPA[27] in seven specimens of lesional skin from subjects with BD and in eight of the controls. To the tissue sections the specific antibody was applied at room temperature and after 5 min the fibrin film was placed over the section and processed as previously mentioned.

TABLE 2. Cutaneous and plasmatic fibrinolytic activity and cutaneous fibrinolytic potential after histamine injection in nine patients affected with Behçet's disease and in controls. All patients, except Nos 4, 6 and 7 had had an episode of thrombophlebitis of lower limbs

	Patients					Controls				
		CFAI					CFAI			
Patient	CFAP	1 h	24 h	72 h	ELT h	CFAP	1 h	24 h	72 h	ELT h
1	++	+++	+	0	10	++	+++	+	++	5
2	++	++	0	0	6·3	++	+++	+	++	6
3	++	+++	+	0	7	++	+++	+	++	6
4	++	+++	+	++	5	++	+++	+	++	2
5	+	++	0	0	7	++	+++	+	++	4
6	++	++	+	+	9·3	++	+++	+	++	3
7	++	+++	+	+	4·3	++	+++	+	++	2
8	++	+++	+	0	8	++	+++	+	++	7
9	++	+++	0	0	8	++	+++	+	++	4

CFAP, cutaneous fibrinolytic activity perilesional; CFAI, cutaneous fibrinolytic potential after histamine injection; ELT, euglobulin lysis time test.

A statistical analysis of the euglobulin lysis time (ELT) between BD patients and controls was made using the Student's t-test.

RESULTS

The results for the plasma and cutaneous FA are shown in Table 2. The plasma FA was lower in patients with BD ($7{\cdot}21 \pm 1{\cdot}86$) than in controls ($4{\cdot}33 \pm 1{\cdot}8$) ($P < 0{\cdot}01$). The cutaneous FA was similar to the control group in eight of the nine patients with BD.

The cutaneous FA 1 h after the injection of histamine was increased in all the controls and was also increased in six of the nine patients with BD, the other three had only a minimal increase.

The cutaneous FA was slightly reduced (+) after 24 h in all the controls and in six of the nine patients with BD. In the other three patients there was no detectable FA. After 72 h all the controls showed levels of cutaneous FA that were similar to those seen before the injection of histamine. Six of the nine BD patients had no detectable cutaneous FA, two had a reduced amount and one was normal. The three patients with BD in which there was restoration of cutaneous FA 72 h after injection of histamine were those who had not had previous thrombotic episodes.

Monoclonal antibodies directed against tPA inhibited the cutaneous FA in both patients and controls, whereas anti-UK antibodies had no detectable effect on cutaneous FA.

DISCUSSION

The plasma FA as determined by ELT showed that it was significantly reduced in patients with BD. This suggests that low levels of circulating plasminogen activators are present in these patients. The perilesional cutaneous FA was similar to that seen in control skin before the intradermal injection of histamine,[28–29] and 72 h afterwards the cutaneous FA was, in general, reduced or absent in the patients with BD. The only ones who showed partial or total restoration of the cutaneous FA were those who had no previous thromboses. The cutaneous FA was shown, by the use of specific antibodies, to be due to the release of tPA and not UK.

We suggest that the cutaneous fibrinolytic potential, tPA dependent, is of help in the prognosis in Behçet's disease and in monitoring endothelial injury. Also treatment with drugs that enhance fibrinolysis such as stanozolol, defibrotide and glycosaminoglycans, are of help particularly in those patients with a history of thromboses and who show reduced levels of plasma and/or cutaneous FA.

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Pigmented spindle cell naevus and its variants: distinction from melanoma

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SUMMARY

We report seven examples of pigmented spindle cell naevus (PSCN) and variants, emphasizing their histopathologic differentiation from malignant melanoma (CMM). Confusion with CMM may occur because of upward migration (pagetoid spread) of cells, prominent lateral extension of lentiginous melanocytic hyperplasia, and cytological atypia in spindle cell naevi. However, these proliferations are usually associated with a symmetrical and orderly growth pattern, confinement of the pagetoid spread to the lower epidermis, 'maturation' of the dermal component, and lack of marked cytological atypia.

Pigmented spindle cell naevus (PSCN) was described in 1975[1] as a distinct variant of spindle and epithelioid cell (Spitz) naevus, characterized by prominent pigment production, relatively flat topography, and fascicles of uniform spindle cells generally limited to the epidermis and papillary dermis.[1–5] In addition to typical PSCN there are superficial pigmented spindle cell naevi that show varying degrees of architectural and cytologic atypia and/or overlap with Spitz naevus. Although possible confusion of these pigmented spindle-cell proliferations with cutaneous malignant melanoma (CMM) has been recognized[1–5] these spindle cell naevi continue to be mistaken for CMM. We report seven examples of PSCN and its variants with emphasis on their differentiation from CMM.

METHODS

Seven examples of pigmented spindle cell naevus were selected from our files over the past year and the clinical and histopathological features reviewed. The criteria for selection included: relatively flat lesions confined to the papillary dermis; predominance of spindle cells; presence of significant melanin within the lesion; and the exclusion of those with typical features of dysplastic naevi.

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One lesion represented a classical PSCN while the other six were classified as variants of PSCN based on architectural and/or cytological atypia, as compared to typical PSCN; and the presence of some features of Spitz (spindle and epithelioid cell) naevus, or both. The latter six lesions were selected because they were originally diagnosed as melanoma or considered to be markedly atypical by the referring pathologist.

RESULTS

Clinical features

The clinical features are outlined in Table 1. Six of seven lesions were located on the extremities of young adults. Almost all the lesions were flat or slightly elevated and deeply pigmented. Both

TABLE 1. Clinical features

Patient	Age	Sex	Anatomic site	Clinical characteristics	Duration of lesion	Clinical diagnoses
1	6	M	Forearm	Macular, less than 5 mm diameter dark, recently enlarged in size	Unknown	R/O melanoma
2	38	M	Left arm	Slightly elevated, dark brown, 3 mm diameter	Unknown	Melanoma
3	32	M	Left thigh	Relatively flat, dark, unevenly pigmented, 10 mm diameter recently changed	Unknown	Melanoma, dysplastic naevus
4	31	F	Right posterior thigh	Recent changes darker, larger	Many years	Atypical naevus or melanoma
5	28	M	Right dorsal foot	Dark mole, no recent change	Many years	Acral lentiginous melanocytic hyperplasia
6	29	F	Extensor aspect, left arm	Round, 3·5 mm in diameter, slightly raised, sharp regular borders, uniform very dark brown, no erythema	9 months	Active junctional naevus vs. superficial spreading melanoma
7	39	F	Right buttock	Roundish, sharp but slightly scalloped borders, slight elevation, uniform blue–black coloration, 4·5 mm diameter, recent enlargement.	Unknown	Dysplastic naevus, R/O melanoma

TABLE 2. Histopathological characteristics of PSCN and PSCN variants

	Histopathological findings in typical PSCN	Histopathological findings in atypical variants of PSCN*	Histopathological findings in variants of PSCN with features of Spitz naevus*
Architectural	Relatively flat Well circumscribed Symmetric Fascicles of uniform spindle cells entirely intraepidermal or extending into papillary dermis Epidermal hyperplasia, slight to moderate Prominent numbers of melanin-laden macrophages in papillary dermis Some upward migration of naevomelanocytes as single cells (pagetoid spread) Usually no laterally extending single cell hyperplasia (lentiginous melanocytic hyperplasia)	Greater tendency to upward migration of single cells (pagetoid spread) Peripherally extending single cell hyperplasia (lentiginous melanocytic hyperplasia)	Fascicles of plump, almost epitheloid spindle cells Clefting about junctional nests
Cytological	Closely apposed, slender spindle cells Generally no cytological atypia Delicate chromatin patterns in nuclei Inconspicuous nucleoli Occasional mitotic figures	Varying degrees of cytological atypia of spindle cells (Nuclear enlargement, pleomorphism, prominent nucleoli, hyperchromatism) Tendency to epitheloid cell characteristics Occasional mitotic figures	Cytological atypia may be present Components of epitheloid cells
Host response	Little or no lymphoid response.	Lymphoid infiltrates	Lymphoid infiltrates

* Characteristics similar to PSCN with the exception of the following.

regular and irregular borders were noted and the diameter of the lesions ranged from 3 to 10 mm. In general largely because of their dark colour, all were considered as atypical and possibly malignant. Although most of the patients could not recall how long the lesion had been present, they had noted a recent change in the lesion. There was no history of dysplastic naevi or CMM in any of the patients.

Histopathological findings

The histopathological characteristics of typical PSCN (case 1) and the PSCN variants are outlined in Table 2. The overall configuration of the lesions was that of a relatively flat naevus with symmetrical and orderly arrangement of fascicles of spindle cells confined to the epidermis and papillary dermis (Figs 1–5). Lentiginous melanocytic hyperplasia (single cell hyperplasia along the basal layer) was observed in every specimen, particularly in the last five cases (Figs 3, 4 and 5) and a pagetoid spread of naevomelanocytes was present in six cases but in general was

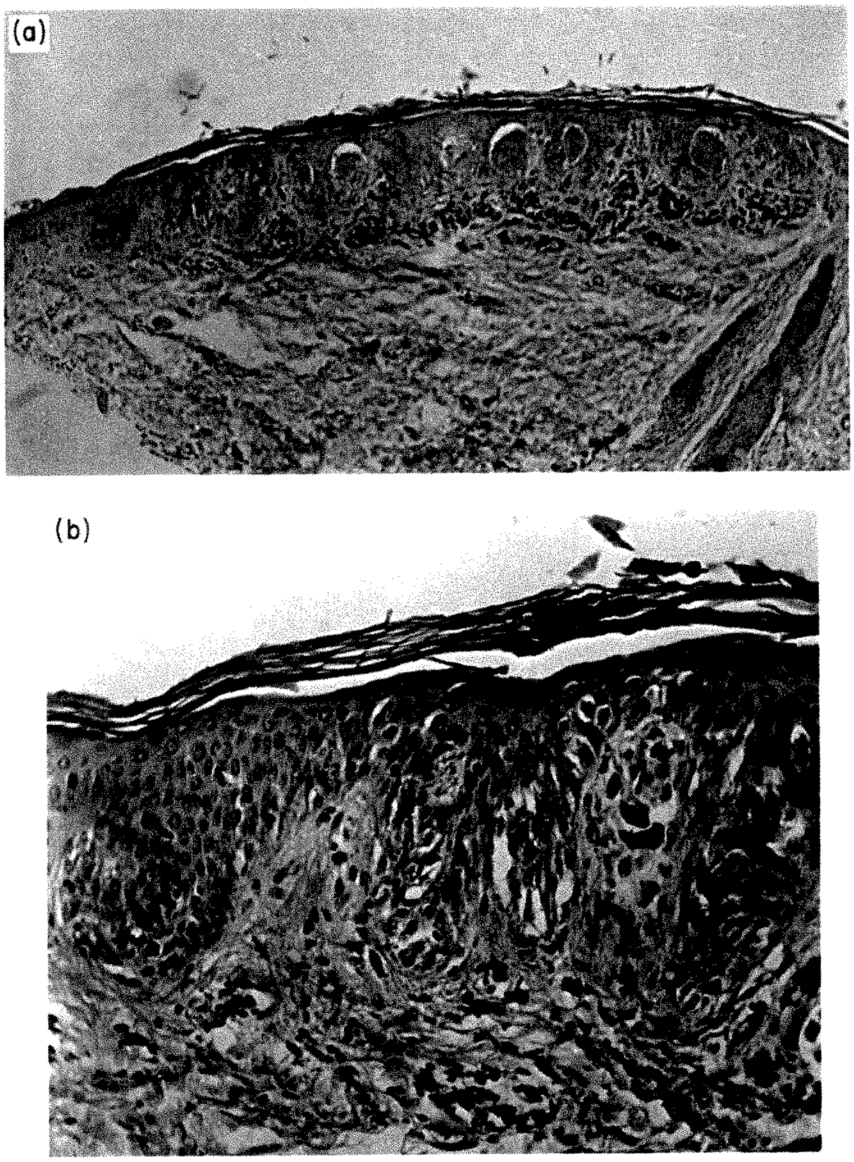

FIGURE 1. Typical PSCN (case 1). (a) Slightly elevated profile, limited to papillary dermis (H and E, × 31). (b) Fascicles of pigmented spindle cells are present along with a tendency for upward migration of single cells (H and E, × 200).

limited to the lower half of the epidermis (Figs 3–5). Most of the atypical variants had a poorly-circumscribed peripheral margin, and lateral extension of the junctional component beyond the central dermal naevus (Figs 3a and 5a).

Cytologic atypia was observed in the six variants with a tendency to nuclear enlargement, pleomorphism and prominent nucleoli. In general the spindle cells had slightly eosinophilic or

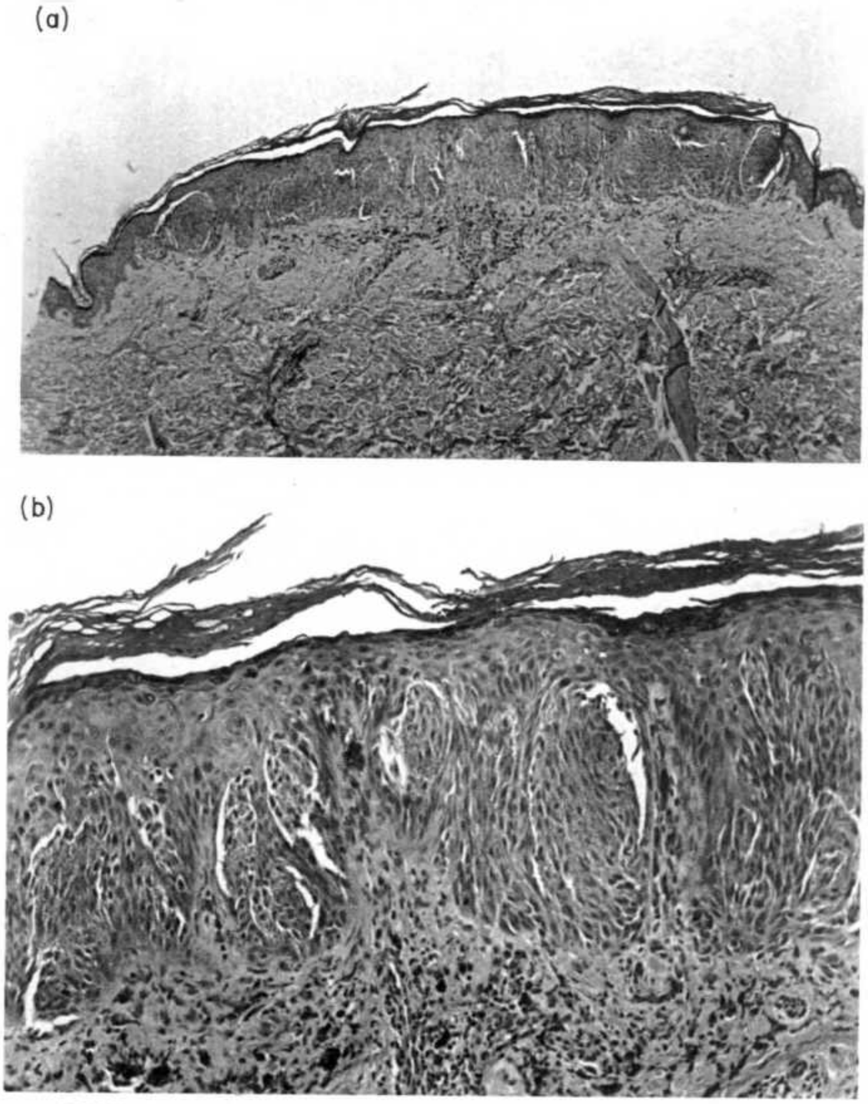

FIGURE 2. PSCN with cytological atypia (case 2). (a) Circumscribed and symmetrical architecture maintained (H and E, × 31). (b) Fairly uniform fascicles of spindle cells (H and E, × 125).

amphophilic cytoplasm with a minority of cells containing abundant melanin. There was also a tendency to have epithelioid cell components in the atypical, and particularly the Spitz variants. The dermal component consisted of small fascicles of spindle cells and single cells. Many of the deeper cells exhibited 'maturation' change with smaller nuclei and cell masses (Figs 3a, 4b, and 5b).

The host response consisted of a perivascular lymphoid infiltrate of variable density, (Figs 4a and 5b), and melanin-containing macrophages that were frequently perivascular (Fig. 3b) but also in a diffuse pattern in the papillary dermis (Fig. 1a) with in some cases fibroplasia. In general

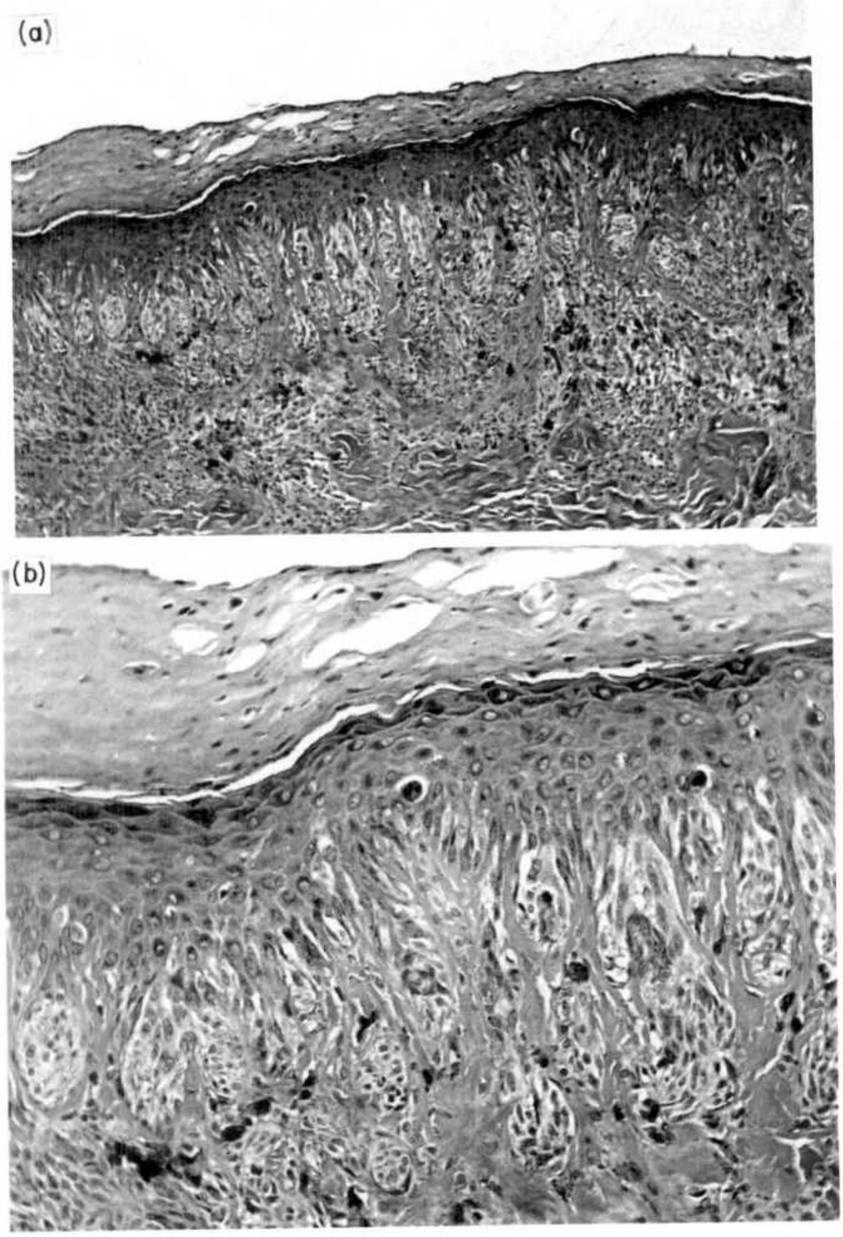

FIGURE 3. PSCN with architectural and cytological atypia (case 5). (a) Prominent single cell hyperplasia along basal layer and some upward spread but generally confined to the lower half of the epidermis (H and E, × 79). (b) Vertically oriented fascicles of plump spindle cells with slight cytological atypia (H and E, × 125).

the character of fibroplasia was non-specific. Other frequently observed features included hyperkeratosis and melanin in the horny layer with slight to moderate epidermal hyperplasia and elongated epidermal rete pegs. There was significant epidermal hyperpigmentation, eosinophilic (Kamino) globules, and clefting around junctional nests.

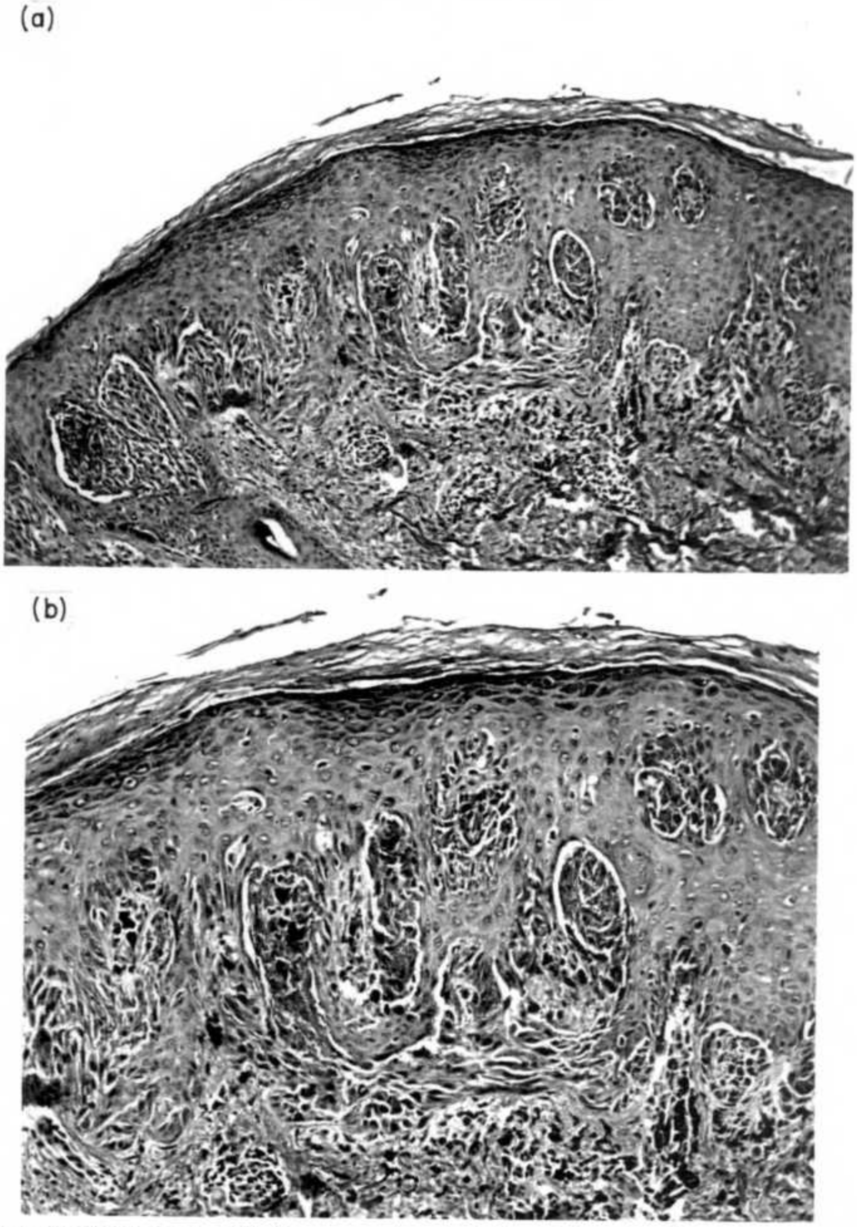

FIGURE 4. PSCN with features of Spitz naevus and cytological atypia (case 4). (a) Significant melanization in papillary dermis (H and E, × 79). (b) A few cells are scattered throughout the epidermis. Fascicles of slightly atypical spindle cells are present (H and E, × 125).

DISCUSSION

Careful correlation of histopathological features and other information such as morphometry, DNA content, antigenic markers, etc. with long-term biological behaviour is required for proper diagnosis of naevomelanocytic proliferations. A particular problem in diagnosis has been

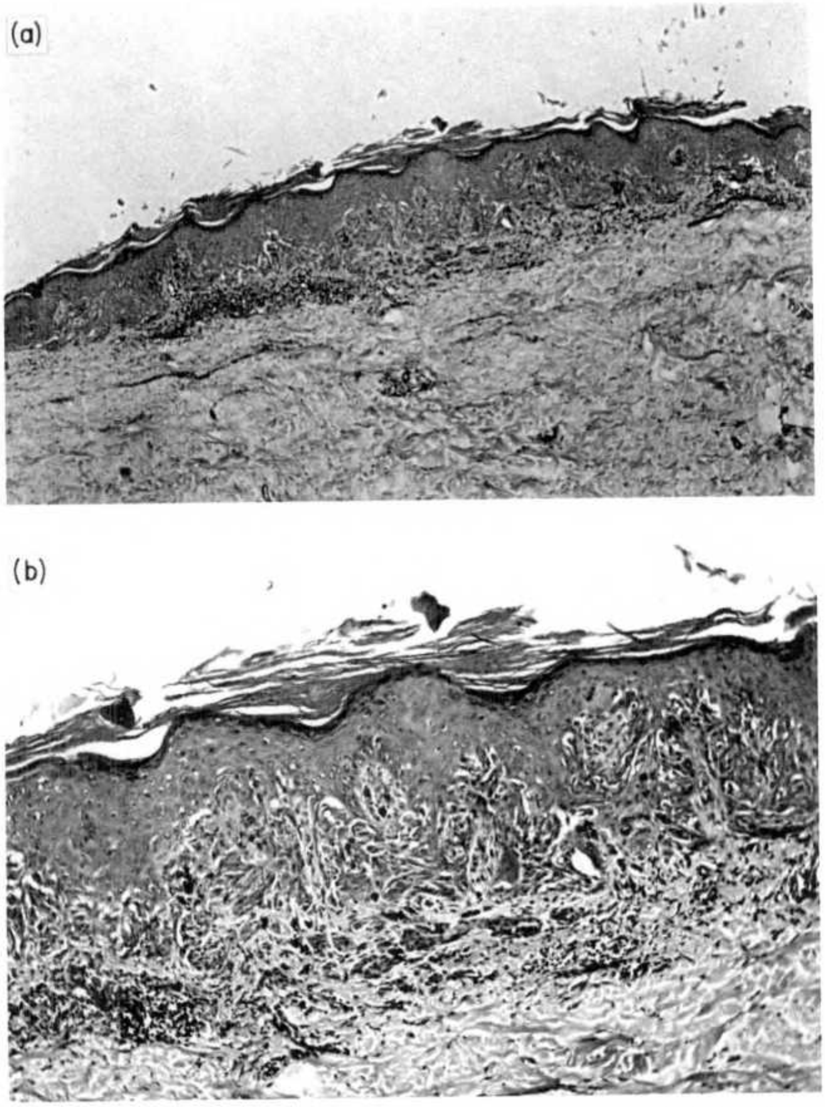

FIGURE 5. PSCN with features of Spitz naevus and with architectural and cytological atypia (case 7). (a) Relatively flat profile, fairly symmetrical pattern, limitation to papillary dermis (H and E, × 31). (b) Fascicles of plump spindle cells with slight atypia and a perivascular lymphoid response (H and E, × 79).

the difficulty of separating Spitz naevus and PSCN from CMM. PSCN and its variants are often misdiagnosed as CMM by referring pathologists and because of this we have outlined the histological features of PSCN, both typical and atypical types, as compared to those of CMM (Table 3).

Typical PSCN as described in case 1 and the original report[1] is an uncommon lesion (Reed, personal communication) in contrast to the atypical and Spitz type variants of PSCN. These variants have both architectural and cytological abnormalities in contrast to typical PSCN (Table 3) which consist primarily of lateral single cell hyperplasia and varying degrees of

TABLE 3. Histopathological similarities and differences between PSCN and melanoma

	Features that may be shared by both PSCN and melanoma	Differences between PSCN and melanoma	
		PSCN	Melanoma
Architectural	Lack of sharp lateral demarcation Lateral extension Lentiginous melanocytic hyperplasia Pagetoid spread	Symmetry	Asymmetry
		Pagetoid spread usually limited to lower half of epidermis	Pagetoid spread affecting all levels of epidermis
		Perivascular lymphoid infiltrates	Band-like lymphoid infiltrates
		Maturation of dermal component	Lack of maturation
Cytological		Lack of fully-evolved cytological atypia	Fully-evolved cytological atypia
		Relatively uniform cytological characteristics of spindle cells	Pleomorphism
		Minimal mitotic activity	Mitotic activity greater

cytological atypia. Plump or slightly epithelioid cells may be present resulting in variable overlap with the Spitz naevus. In general the difference between typical PSCN, its variants, and other spindled proliferations including dysplastic naevi have not been appreciated in the literature (Reed, personal communication) and PSCN and its variants continue to be frequently misdiagnosed as melanoma. The reasons for this, in our opinion, are the lack of familiarity with the spectrum of PSCN by many histopathologists, the difficulty of spindle cell proliferations in general, and the greater emphasis placed on architectural rather than cytological abnormalities.[6] Although these spindle-cell proliferations may have pagetoid spread, varying degrees of cytological atypia, and some degree of host response, they have in general a symmetrical orderly growth pattern and lack the marked atypia seen in melanoma. The absence of a vertical growth phase (with the formation of an expansile nodule) is further evidence against the diagnosis of melanoma.

There is limited information about the biological behaviour of pigmented spindle-cell proliferations and in one series the follow-up was obtained in 38 out of 90 cases and ranged from 6 to 40 months (mean 14 months).[5] No evidence of recurrence or metastasis was reported. However, long-term follow-up (at least 5 years) is needed to know about the natural history and the benign nature of these lesions.

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Mucosal involvement in systemic and chronic cutaneous lupus erythematosus

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SUMMARY

The prevalence and pattern of mucosal involvement in 121 patients with lupus erythematosus (LE) was investigated. Fifty-three patients had systemic LE (SLE) and 68 patients had chronic cutaneous LE (CCLE). Twenty-one per cent (11/53) of patients with SLE and 24% (16/68) of patients with CCLE had signs of mucosal involvement, but the pattern of involvement differed in the two groups. Nasal mucosal lesions were a feature in 2% (1/53) of patients with SLE and 9% (6/68) of patients with CCLE. Hyperkeratotic lichen planus-like plaques on buccal mucosa and the palate occurred in 9% (6/68) of patients with CCLE and 4% (2/53) of patients with SLE. Episcleritis occurred in 9% (5/53) of patients with SLE and was not seen in CCLE. Erythematous plaques on the lower eyelids were present in 6% (4/68) of patients with CCLE and these were associated with conjunctival scarring in two patients. Vulval lesions were present in 5% (2/42) of female patients with CCLE. Oral plaques may occur when the disease is relatively quiescent elsewhere. The prevalence of mucosal involvement in lupus is underestimated as the lesions may be asymptomatic.

The prevalence of mucosal involvement in patients with systemic lupus erythematosus (SLE) or chronic cutaneous lupus erythematosus (CCLE) is uncertain. In SLE the reported prevalence of oral manifestations such as acute ulceration or chronic mucosal plaques varies from 9 to 45%.[1–3] Oral and nasopharyngeal ulcerations are one of the criteria proposed by the American Rheumatism Association (ARA) for identifying patients with systemic lupus erythematosus,[4] but frequently clinical findings in the mucosa are poorly documented and no distinction is made between acute mucosal ulceration and ulceration within atrophic chronic mucosal plaques. The nasal septum,[5–7] vagina[6] and conjunctiva[8] may also be involved in SLE.

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The reported prevalence of oral manifestations in CCLE ranges from 3% to more than 20%.[3,9,10] Perianal mucosal lesions[11] and conjunctival lesions[12] have been described in CCLE, but the frequency of mucosal involvement in CCLE is unknown. In this study we have investigated the prevalence and pattern of mucosal involvement in a total of 121 patients, 53 with SLE and 68 with CCLE.

METHODS

Patients

Informed consent was obtained from the patients and the study had the approval of the Oxford Research Ethics Committee. The majority of patients with SLE were recruited from the Nuffield Department of Medicine, Oxford, or the Rheumatology Department, Oxford, and patients with CCLE were recruited from Departments of Dermatology in Oxford and High Wycombe.

Systemic LE. Fifty-three patients with SLE were included in the study. The patients satisfied the revised criteria of the American Rheumatism Association for the diagnosis of SLE.[4] There were five males (age range 27–71; mean age 43) with a mean age at disease onset of 31 years and 48 females (age range 13–83; mean age 42) with a mean age at disease onset of 30 years. The patient details are shown in Table 1 and clinical features in Figure 1.

Chronic cutaneous LE. Sixty-eight patients with histologically confirmed cutaneous LE were included in the study. Forty-eight had localized disease (chronic discoid lesions above the chin only) and 20 had generalized disease (chronic discoid lesions below the chin with or without lesions present above this level). There were 42 females (age range 22–83; mean age 53) with a mean age at disease onset of 38 years and 26 males (age range 29–78; mean age 50) with a mean age at disease onset of 39 years. The patient details are shown in Table 1 and clinical features in Figure 1.

Clinical assessment

A detailed history was taken, particularly of symptoms involving the eyes, nose, lips, mouth and perineum. All mucosal surfaces and the perineum were examined. Patients with oral signs (five SLE and nine CCLE) were assessed by an oral surgeon (RJ) and when possible the clinical

TABLE 1. Patient details

	SLE ($n=53$)	CCLE ($n=68$)
Sex		
Male	5	26
Female	48	42
Age		
Male	27–71 (mean 43)	29–78 (mean 50)
Female	13–83 (mean 42)	22–83 (mean 53)
Disease duration	3 months–31 years (mean 10 years)	6 months–54 years (mean 12 years)

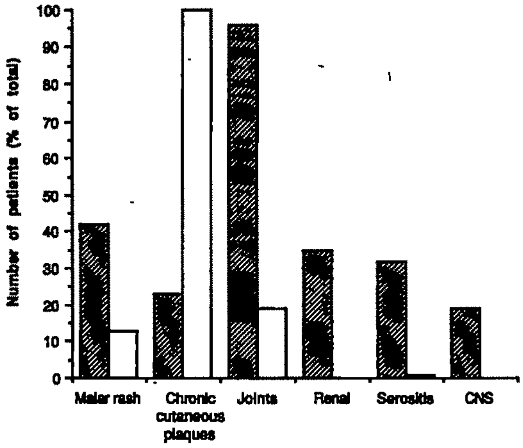

FIGURE 1. Clinical features in 53 patients with SLE and 68 patients with CCLE. ■, SLE; □, CCLE.

diagnosis was confirmed by an oral biopsy which was examined by light microscopy and direct immunofluorescence. Forty-eight patients (18 SLE and 30 CCLE) were referred for full assessment by an ophthalmologist (PAF). The anterior ocular surface was examined using a slit lamp and Schirmer's test was performed to evaluate tear production. A Schirmer's test result of < 10 mm in 5 min in both eyes was regarded as a significant reduction in tear production.

RESULTS

The distribution of mucosal lesions in SLE and CCLE is shown in Figure 2.

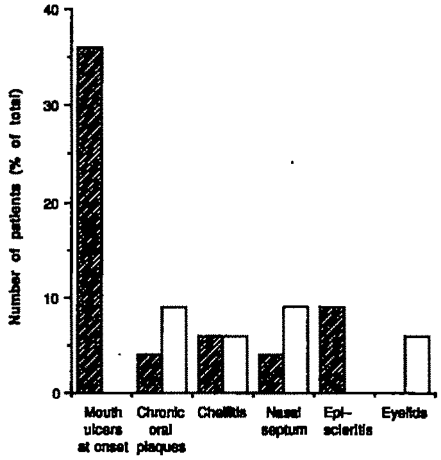

FIGURE 2. Mucosal lesions in 53 patients with SLE and 68 patients with CCLE. Clinical mucosal involvement was present in 21% of 53 patients with SLE and 24% of 68 patients with CCLE, but the pattern of involvement differed in these groups. ■, SLE; □, CCLE.

TABLE 2. SLE: patients with signs of mucosal involvement

Sex	Age	Disease duration (years)	Systemic therapy (daily)(mg)	Renal disease	Photo sensitivity	Chronic cutaneous LE	Episcleritis	Cheilitis	Chronic oral lesions	Acute palatal ulcers	Nasal septal erythema
F	21	3 months	None		+	+				+	
F	23	9 months	None		+		+				
F	28	7 years	Pred. 2·5 Aza. 100 H-Cq. 200		+						+
F	44	3 years	Pred. 5	+	+		+				
M	30	10 years	None		+	+		+			
F	39	19 years	Pred. 6		+	+		+	+		
F	70	31 years	Pred. 5		+	+		+			
F	54	13 years	Pred. 20 Aza. 100	+	+		+				
F	59	23 years	Pred. 2·5		+		+				
M	57	3 years	Pred. 7·5 Aza. 100		+	+	+				
F	22	10 years	Cq. 50		+	+			+	+	

Pred., Prednisolone.
Aza., Azathioprine.
H-Cq., Hydroxychloroquine.
Cq., Chloroquine.

SLE

Twenty-one per cent of SLE patients (11/53) had signs of mucosal involvement, and the clinical details are given in Table 2.

Nasal mucosa. One patient had a history of recurrent nasal mucosal ulceration but the SLE was in remission and there were no mucosal symptoms or signs. One patient with mild SLE had nasal mucosal irritation and soreness with persistent erythema and crusting of the anterior nasal septum. Septal perforation was not detected in any of the patients with SLE.

Oral mucosa

(a) Cheilitis. Asymptomatic cheilitis was observed in 6% (three patients) who were all photosensitive. The lips had a silvery appearance, there was erythema and scaling and the vermilion border was ragged and blurred.

(b) Ulcers. Mouth ulcers were a feature in 36% (19 patients). Seventeen of these patients gave a history of painful mouth ulcers, which were present during the acute phase of their disease, but it is not possible to be certain of the morphology or pathogenesis of this ulceration. Asymptomatic ulceration was detected in one patient, who was seen at the onset of SLE. This 21-year-old female had a large, well-demarcated, erythematous ulcer in the midline of the hard palate surrounded by a raised, white, hyperkeratotic border. There were also focal areas of gingivitis and ulceration behind the upper teeth. A further patient with inactive SLE but localized cutaneous discoid LE had painful ulceration in association with chronic mucosal plaques (Fig. 3) (see below).

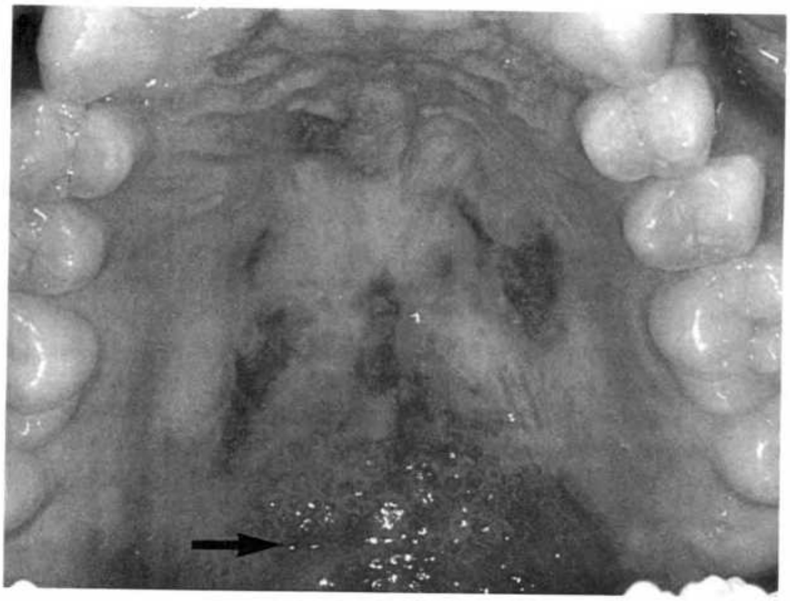

FIGURE 3. SLE. Discoid plaque on the soft palate and focal ulceration with scarring of the hard palate. The plaque has a distinctive 'honeycomb' appearance [→]. The patient has inactive systemic disease and 3 small cutaneous discoid lesions.

TABLE 3. CCLE: patients with signs of mucosal involvement

Sex	Age	Disease duration (years)	Localized CCLE	Generalized CCLE	Therapy (daily) (mg)	Photo sensitivity	Eyelid lesions	Conjunctival scarring	Cheilitis	Chronic buccal plaques	Chronic palatal plaques	Nasal septal plaques	Genital lesions
F	57	13	+		Topical	+						+	
M	29	14		+	Topical							+	
M	59	25		+	Topical							+	
M	44	9	+		Topical	+				+		+	
F	65	37	+		Topical		+	+	+			+	
F	54	24	+		H-Cq.400	+						+	
F	65	7	+		None					+			+
F	59	15	+ (inactive)		None		+			+	+		
F	55	22		+	Mep. 100	+				+			
F	47	6	+		Topical	+				+			
M	44	7	+		Topical	+			+	+	+		
M	32	7	+		Topical	+			+				
M	44	2	+		Topical	+			+				
M	68	54		+	Topical	+	+	+					
F	23	2	+		Topical		+						
F	67	37		+	Topical	+							+

Mep., Mepacrine.
H-Cq., Hydroxychloroquine.

(c) Chronic plaques and lichen planus-like lesions. One female patient with a past history of systemic disease and mild chronic cutaneous disease on the nose and ears had a 10-year history of persistent, widespread painful involvement of the hard palate, soft palate and buccal mucosa, which had necessitated treatment with chloroquine. There was a well demarcated, erythematous plaque on the soft palate. Short white hyperkeratotic strands running across the surface with an annular configuration gave the whole plaque a characteristic 'honeycomb' appearance (Fig. 3). There was ulceration and scarring of the hard palate with focal hyperpigmentation secondary to prolonged treatment with chloroquine. The buccal lesions were sharply demarcated, ulcerated and surrounded by a hyperkeratotic border.

Another patient, who also had cutaneous discoid LE in addition to well-controlled SLE, had an asymptomatic small ill-defined, lacy white lesion in the lower buccal sulcus. This was clinically indistinguishable from lichen planus.

(d) Non specific lesions. One patient had diffuse hyperkeratosis and uncomfortable ulceration on the lower lip probably secondary to recurrent trauma and another patient with vasculitis had recurrent small lesions resembling aphthous ulcers.

(e) Tongue. No abnormalities were detected.

Ocular mucosa. Nine per cent (5/53) had episodes of episcleritis with redness of the globe and this was the presenting feature in one of these patients. Lid margin plaques or conjunctival scarring were not seen in patients with SLE. Eleven per cent (2/18) had a significantly reduced Schirmer's test (< 10 mm in 5 min in both eyes) and one of these patients was asymptomatic.

Genital mucosa. Symptoms were prominent in one patient with Sjogren's syndrome who complained of vaginal dryness and discomfort, but there were no specific lesions.

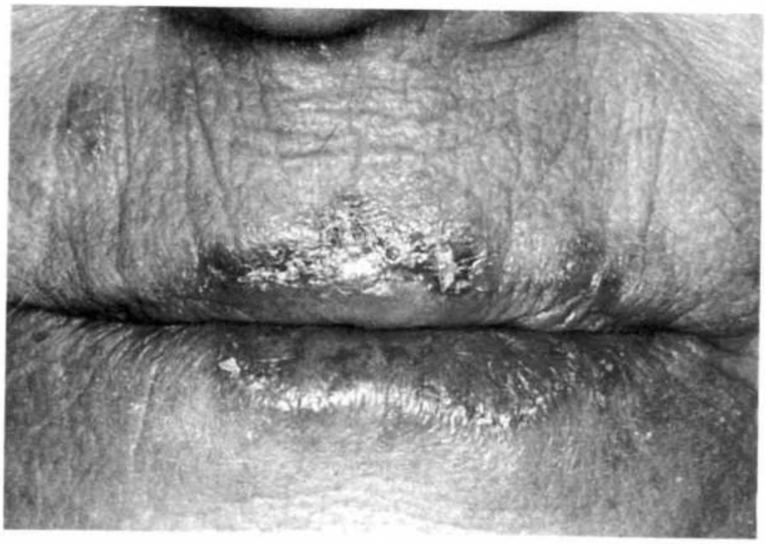

FIGURE 4. CCLE. Well defined plaques on the vermilion border.

CCLE

Twenty-four per cent of CCLE patients (16/68) had signs of mucosal involvement and the clinical details are shown in Table 3.

Nasal mucosa. Symptomatic nasal septum involvement with persistent erythematous plaques, crusting or scarring of the anterior nasal was present in 9% (six patients), all of whom had active cutaneous disease. Septal perforation was not detected.

Oral mucosa

(a) Cheilitis. Cheilitis was a feature in 6% (four patients) all of whom were photosensitive. Well defined small plaques on the vermilion border (Fig. 4) were present in one of these patients; two patients had asymptomatic small plaques on the lips and the fourth had diffuse erythema and scaling of upper and lower lips. These patients had active disease elsewhere.

(b) Chronic plaques or lichen planus-like lesions. Chronic plaques or lichen planus-like lesions were present in 9% (six patients). The buccal plaques had a well-defined, irregular scalloped white margin with radiating peripheral short white striae and telangiectasia (Fig. 5), central erythema which extended to close to the edge of the lesion and, in some cases, central clearing and fibrosis.

In two of the patients there were also palatal lesions. In one of these patients the plaque was situated at the junction of the hard and soft palate extending onto both (Fig. 6a), but in the other the lesion was restricted to the soft palate (Fig. 6b). These chronic plaques also had the striking

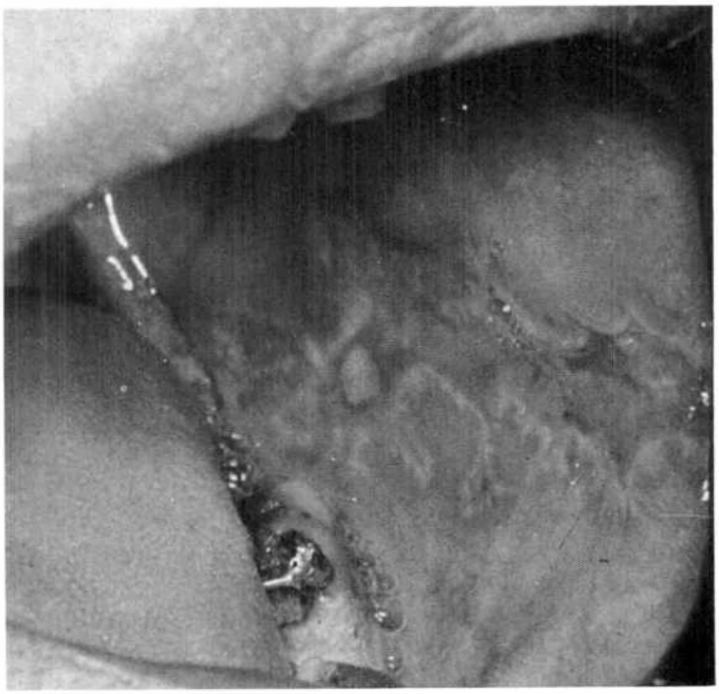

FIGURE 5. CCLE. Ulcerated discoid plaque on the left buccal mucosa. The hyperkeratotic border has a well defined irregular, 'scalloped' appearance with radiating striae and peripheral telangiectasiae.

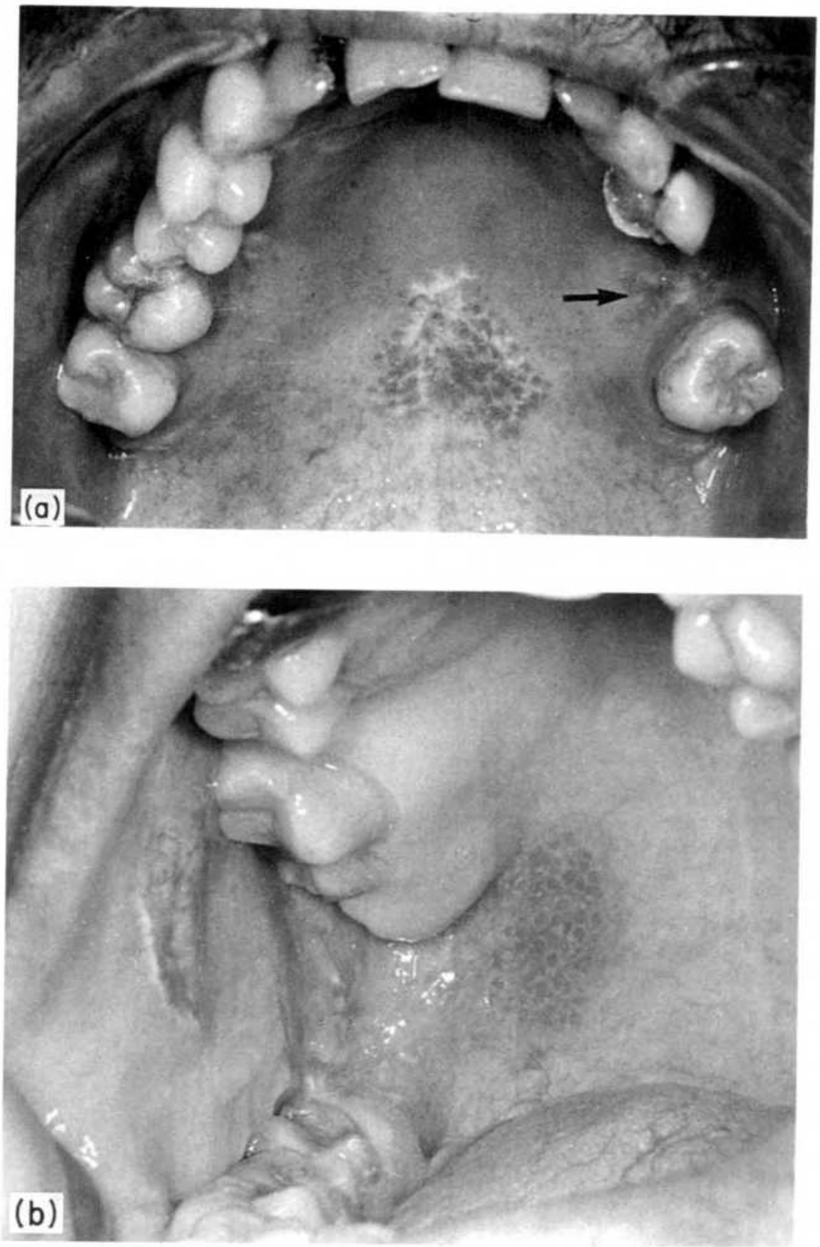

FIGURE 6. (a) CCLE. Chronic discoid plaque. The lesion involves both the keratinized hard and the non-keratinized soft palate and is characterized by an interlacing network of hyperkeratotic white strands overlying an erythematous base giving a honeycomb appearance. There is also a focal erythematous plaque on the upper alveolar process at the site of a missing tooth [→]. (b) CCLE. Chronic discoid plaque on the soft palate. The 'honeycomb' appearance is characteristic. There is less hyperkeratosis in this lesion on the soft palate than there is on the hard palate. There is also a buccal plaque.

'honeycomb' appearance with a well-defined meshwork of raised hyperkeratotic white strands encircling zones of punctate erythema. Hyperkeratosis was more marked in the lesion on the hard palate. Focal erythematous plaques on the alveolar processes at the site of missing teeth (Fig. 6a) were detected in several patients and one patient had additional lesions on the floor of the mouth. Lesions clinically indistinguishable from lichen planus were present on the buccal mucosa of one patient.

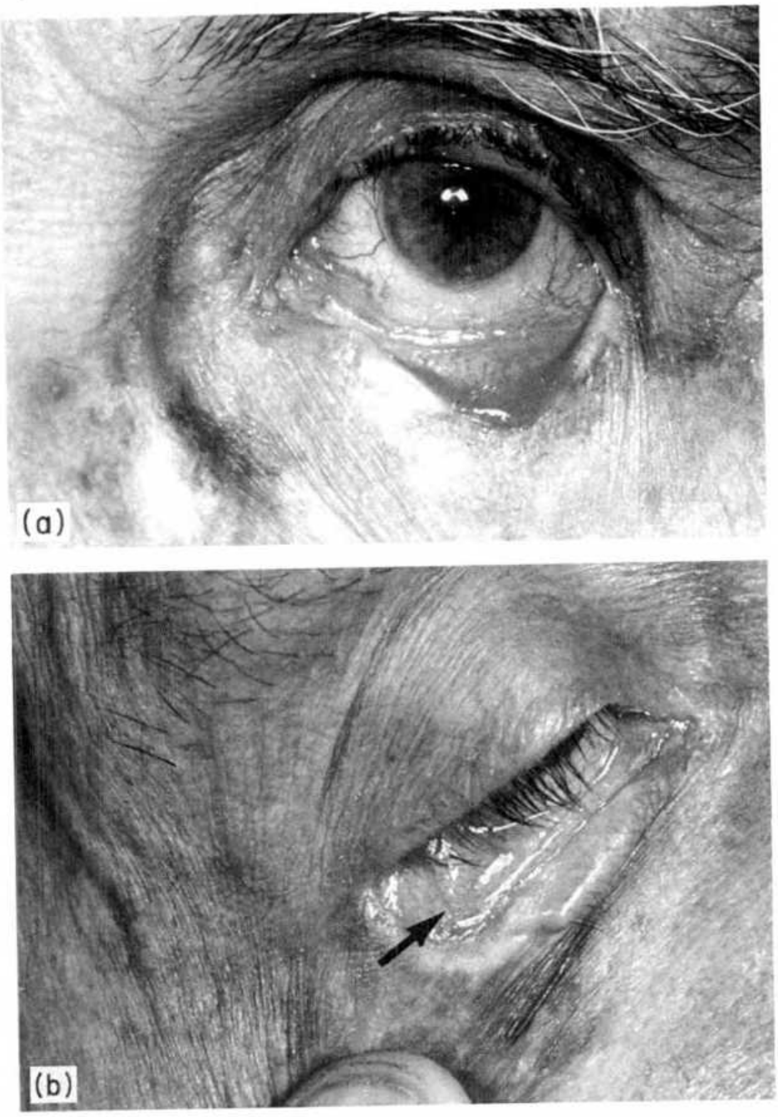

FIGURE 7. (a) CCLE. The patient has had active cutaneous and mucosal disease for more than 30 years. There is scarring of the central portion of the lower lid and tarsal conjunctival involvement. There were similar changes in the other eye. Cutaneous hypopigmentation and hyperpigmentation adjacent to the medial canthus are the consequence of chronic cutaneous involvement. (b) CCLE. The patient has had active cutaneous and mucosal disease for more than 30 years. There is scarring of the lateral portion of lower lid, loss of lashes and conjunctival scarring with symblepharon [→]. There were similar changes in the other eye. Hyperpigmentation below the eye is secondary to cutaneous disease.

The intraoral lesions were asymptomatic in five patients, but painful ulceration within the buccal plaques was a problem in one patient. The oral involvement did not bear any relationship to the severity or activity of cutaneous disease. In five out of six patients with chronic mucosal lesions, the skin disease was well controlled or minimal. One patient had active localized discoid LE.

(c) Tongue. No abnormalities were detected.

Ocular mucosa. Well-defined, erythematous plaques were present on the lower eyelid in 6% (four patients). One patient had unilateral lid involvement, but the others had symmetrical bilateral involvement. The lid lesions were asymptomatic in two patients. In two of the patients there was no associated lid scarring, the plaques responded to topical corticosteroids but tended to recur and were thought to be a manifestation of early lid CCLE. In the other two patients, both with long-standing disease (> 30 years), the plaques involved the lower lid margins bilaterally and were associated with marked symmetrical erosion of the lid margins, loss of lid tissue, disorganization of the mucocutaneous junction, loss of lashes and tendency to eversion of

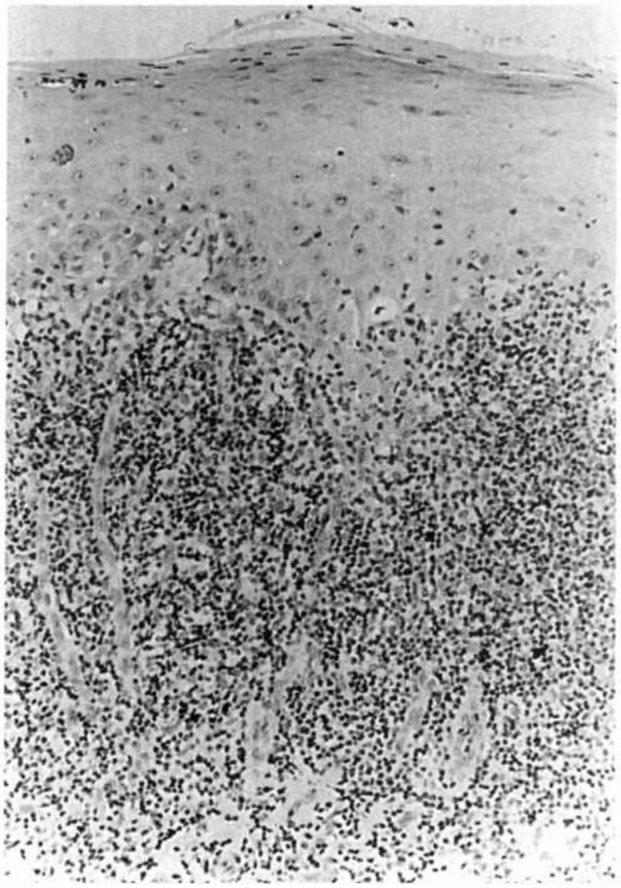

FIGURE 8. Biopsy from a chronic discoid plaque on the buccal mucosa showing a lichenoid lymphocytic infiltrate along the basement membrane zone with liquefaction degeneration of basal cells and colloid bodies in the underlying submucosa. (H & E; original × 127·5).

the lower lid (ectropion) (Fig. 7a, b). In both these patients the lid lesions were locally associated with significant tarsal conjunctival scarring in the lower lid. This produced symblepharon or loss of normal forniceal folding with adhesions, apparent when the lower lid is everted. One of these patients had active localized disease on the face, but in the second patient, there was minimal disease activity apart from the eyelid lesions. Seven per cent (2/30) of CCLE patients had a significantly reduced Schirmer's test but were asymptomatic.

Genital mucosa. Vulval changes were present in 5% (2/42 female patients). In one patient there was erythema and painful ulceration near the vaginal introitus, which healed leaving a pale atrophic scar. The patient did not give consent for a biopsy. Another patient had white lacy changes and erythema on the vulval skin in addition to asymptomatic oral lichen planus-like lesions and biopsies were taken from mouth and vulva (see below).

Histology. Biopsies were taken from the chronic buccal plaques in one patient with SLE and four patients with CCLE and from the lichen planus-like oral lesions in one patient with CCLE and the lichen planus-like vulval lesions in one patient with CCLE.

The histology of the biopsies from the chronic buccal plaques showed parakeratosis and acanthosis with hydropic degeneration of the basal layer and a lichenoid mononuclear cell dermal infiltrate (Fig. 8). The specimens were examined by direct immunofluorescence and granular linear deposition of immunoreactants was detected along the basement membrane zone in all these specimens. C3 was present in two specimens; IgG was present in one specimen; C4 in one specimen; and IgG, C4 and C1q in one specimen.

The biopsy from the lichen planus-like buccal lesion showed a lymphocytic infiltrate but the histology was not diagnostic and immunofluorescence was negative.

The histology of the biopsy of the vulval lesion showed a patchy mononuclear cell infiltrate but the findings were not diagnostic.

DISCUSSION

The prevalence of mucosal lesions in patients with different forms of LE is not clearly documented. In this study we have found clinical mucosal involvement in 21% (11/53) of patients with SLE and 24% (16/68) of patients with CCLE.

Oral ulcers occur more frequently in SLE than in CCLE. Activity of the disease is significantly increased during the time of mucosal ulceration[13] and oral ulceration is included in the revised criteria proposed by the American Rheumatism Association for the identification of patients with SLE.[4] Erythema, petechiae, painless ulceration of the hard palate, mucosal erosions, haemorrhage and gingivitis may occur during acute systemic episodes.[1] Although 35% of the SLE patients (18 patients) gave a history of painful oral ulceration which coincided with the onset of their disease, the pathogenesis of these ulcers is uncertain. The clinical records did not exclude the possibility that aphthous ulceration, candidiasis or herpes stomatitis had contributed to their symptoms.

One patient who was examined at the onset of SLE had a large painless erythematous ulcer on the hard palate and focal gingivitis. The hard palate is a site of predilection for erythematous lesions and painless ulcers in acute flares of SLE,[1,3,13] but because the patient may be unaware of the lesions, they are often overlooked. The pathogenesis of palatal ulceration is unclear. There is no evidence of a necrotizing vasculitis and the histology is similar to that of cutaneous lupus.[13] Treatment with systemic corticosteroids usually leads to rapid healing.[1]

Chronic 'discoid' mucosal lesions occur predominantly on the buccal mucosa, alveolar process, labial mucosa and vermilion border of the lip[3,9] and although they have been described in both SLE and CCLE, we found these lesions more frequently in patients with CCLE. Two SLE patients had chronic mucosal plaques and both also had chronic discoid cutaneous lesions. Neither of the patients had renal involvement and in one of the patients the systemic disease was in complete remission, although there was extensive chronic mucosal involvement. Chronic mucosal LE, like discoid cutaneus LE[14] may mark a subset of SLE patients with a good prognosis.

Chronic buccal plaques were also present in seven patients with CCLE and in six patients they were bilateral. The lesions were present predominantly in patients with mild or inactive cutaneous disease and the histological features were similar to those described in previous studies.[15] The typical oral 'discoid' plaque consists of a central atrophic red area with small white dots and a slightly elevated border zone of irradiating white striae and telangiectasia.[9] Although these buccal lesions have many similarities to atrophic lichen planus, the well-defined irregular scalloped outline, short white peripheral striae and erythematous central zone extending close to the margin are helpful distinguishing features. Chronic plaques of LE on the palate have a characteristic 'honeycomb' appearance (Fig. 6) and the site of the lesion may also point to the diagnosis. Lichen planus does not usually involve the anterior part of the buccal mucosa without also involving the posterior surface; nor does it involve the mucosa of the palate unless it is present extensively in the mouth. Several of our patients had lesions on the alveolar process at the site of missing teeth and this has been observed in previous studies.[3] Similar lesions were seen on the gingivae.

There are differences in the structure of the oral mucosa which relect differences in function.[16] Non-keratinized lining mucosa, which covers the soft palate, lips, cheeks and alveolar processes as far as the gingiva, is predominantly involved by chronic 'discoid' lesions, whereas the keratinized mucosa on the hard palate and gingiva is involved by the acute lesions seen in SLE. In keratinizing epitheliae the cell nucleus is lost during differentiation and there is release of DNA in nuclear fragments. Circulating antibodies to native DNA (nDNA) are found in the majority of patients with SLE, and it has been proposed this serum anti-nDNA binds to nDNA released locally from differentiating keratinocytes.[17,18] This may play some part in the initiation or perpetuation of acute ulcerative lesions on hard palate and gingiva in patients with SLE. The majority of chronic 'discoid' mucosal lesions occur on non-keratinizing mucosa in patients without circulating anti-DNA antibodies. In normal non-keratinizing epithelia, the cells retain their nuclei and probably there is less release of DNA during differentiation.[19] The trigger for the initiation of chronic lesions on non-keratinized lining mucosa is unknown.

Lupus cheilitis is usually associated with photosensitivity and predominantly involves the lower lip. Squamous cell carcinoma may develop in chronic lupus cheilitis, particularly on the lower lip.[9,20,21]

Nasal mucosal involvement occurred in two SLE patients and six CCLE patients. Nasal septum perforation in SLE was first reported in 1969[5] and ulceration and perforation are usually associated with acute exacerbations of SLE.[6,22] There is relative stasis and leakiness within the small capillaries and venules of the septum so that circulating immune complexes are readily trapped in these small vessels and can leak into the surrounding tissue where they may trigger local inflammation and vasculitis.[23] Persistent erythematous plaques, crusting or scarring of the anterior nasal septum was present in 9% (six patients) of the CCLE patients, but the pathogenesis of these lesions is uncertain.

Vulvovaginal inflammation may occur in SLE[1] but candidal infections are common and the prevalence of genital mucosal involvement in patients with LE is unknown. We did not find specific acute ulcerative genital lesions in our patients with SLE but these may be more common when the disease is active. Chronic lesions may occur in the vulva in CCLE but are usually overlooked.[24] Two of our female patients with CCLE had vulval pathology which was clinically consistent with local involvement.

The prevalence of external ocular lesions in patients with LE has not been clearly documented. Episcleritis is associated with immune complex mediated inflammation and vasculitis and may be a feature of diseases such as rheumatoid arthritis or Wegener's granulomatosis in addition to SLE. The pattern of chronic involvement seen in the eyelid and conjunctiva is unique to LE but there are less than 50 reported cases of CCLE involving the eyelid.[8,12,25,26] Specific eye lid lesions were present in 6% (4/69) of patients with CCLE but none of the patients with SLE. Symmetrical involvement of the outer third of the lower lids with erythema and oedema predominated early, but later there may be lid scarring with permanent loss of eye lashes. Initially the conjunctival lesions are well defined erythematous oedematous patches with a rather velvety appearance, but in two patients there was progression to marked conjunctival scarring with symblepharon. In one of these patients, repeated asymptomatic episodes of inflammation and progressive conjunctival scarring had occurred over a period of 40 years. Clinically, there were many similarities to the changes seen in the eye in cicatricial pemphigoid but in cicatrical pemphigoid lid-margin abnormalities are not found. Although there are previous reports of LE involving the conjunctiva,[8,12] conjunctival scarring with symblepharon has not been described. The conjunctival lesions respond to treatment with topical corticosteroids, but as with mucosal involvement elsewhere, lesions may recur.

In conclusion, we have found clinical mucosal involvement in 21% of 53 patients with SLE and 24% of 68 patients with CCLE, but the pattern of involvement differs in these groups. Manifestations of active systemic disease such as mucosal ulceration or episcleritis were uncommon in this series of patients with well controlled disease. Chronic oral mucosal lesions may occur when the disease is relatively quiescent elsewhere and ulceration in an atrophic plaque should be differentiated from the acute ulceration of previously normal mucosa which is associated with active systemic disease. Chronic ocular involvement may lead to conjunctival scarring and symblepharon. The mucosal lesions in LE remit and relapse and as they may be asymptomatic, their prevalence may be underestimated.

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The lupus band test in oral mucosa, conjunctiva and skin

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SUMMARY

The prevalence and clinical significance of subepithelial immunoglobulin and complement deposition (the lupus band) were examined in the uninvolved sun-protected skin of the forearm, the uninvolved sun-protected lip mucosa and sun-protected bulbar conjunctival mucosa in systemic lupus erythematosus (SLE) and chronic cutaneous lupus erythematosus (CCLE). In SLE, linear deposition of an immunoreactant at the BMZ was detected in 32% (6/19) of skin biopsies; 21% (4/19) of lip mucosal biopsies and 42% (5/12) of conjunctival biopsies. There was no significant difference in the sensitivity of the test at different sites in SLE and no correlation between a positive test in skin, lip or conjunctiva and clinical mucosal involvement. In CCLE, linear deposition of an immunoreactant at the BMZ was found in 3% (1/32) of skin biopsies; 3% (1/29) of lip mucosal biopsies and 50% (10/20) of conjunctival biopsies. There was no correlation between a positive test in skin, lip or conjunctiva and clinical mucosal involvement. In the conjunctiva, IgG was present in all but one of the biopsies and was the only immunoreactant in 90% (9/10) of positive CCLE biopsies and 60% (3/5) of positive SLE biopsies. In lupus erythematosus immunoreactants may be deposited in the basement membrane zone beneath non-keratinizing mucosal surfaces of the lip and the eye as well as the skin. In CCLE, the test may be positive in conjunctiva when skin and lip are negative.

Lupus erythematosus (LE) is a multi-system disease of uncertain aetiology with variable clinical features and a prognosis which primarily depends upon the presence or absence of neurological or renal involvement. Circulating antibodies to native DNA (nDNA) are found in the majority of patients with systemic LE (SLE) but only in a minority with the disease limited to the skin (chronic cutaneous LE; CCLE).

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Using direct immunofluorescence, a band of immunoreactants, the 'lupus band', may be detected in the uninvolved skin of up to 80% of patients with SLE, but the test is usually negative in the uninvolved skin of patients with CCLE.[1-3] Deposits of immunoreactants have also been demonstrated in the uninvolved oral mucosa in SLE.[4-6]

The significance of a positive lupus band test (LBT) in the uninvolved skin of patients with SLE is controversial.[3] There is evidence that subepidermal deposits are found most frequently in SLE patients with proliferative glomerulonephritis and hypocomplementaemia,[2,7] but this association and the capacity of a positive test to predict renal disease are disputed.[3] The cutaneous deposits of immunoreactants disappear with therapy and vary in adjacent biopsy sites.[2,8] Therefore a single LBT in skin has limited use as a prognostic indicator and a negative test, particularly in patients taking immunosuppressive therapy, does not exclude renal involvement.[8]

TABLE 1. Clinical and serological features in patients with systemic LE

Patient No.	Sex	Age at onset (years)	Disease duration (years)	Systemic therapy (mg)		ANA
1	F	37	7	Pred. Aza.	8 100	+
2	F	37	14	Pred.	5	−
3	F	35	13	Pred.	10	+
4	F	21	7	Pred. Aza. H-Cq.	2·5 100 200	+
5	F	20	19	Pred.	6	−
6	F	32	10	Pred. Aza.	30 100	+
7	F	23	9 months	None		+
8	F	30	17	Pred. Aza.	15 50	+
9	F	48	15	None	(inactive)	−
10	F	40	13	Pred. Aza.	20 100 mg	+
11	F	54	6	Pred. Aza.	7·5 100	−
12	F	32	12	H-Cq.	400	+
13	F	18	1	Pred. Aza.	12·5 150	+
14	F	45	14	None		+
15	F	39	31	Pred.	5	+
16	F	40	3	Pred.	5	+
17	F	36	23	Pred.	2·5	+
18	F	21	15	None	(inactive)	+
19	M	37	10	None		+
20	M	54	3	Aza. Pred.	100 7·5	+

Pred., Prednisolone. +, Positive or involved.
Aza., Azathioprine. −, Negative or not involved.
Mep., Mepacrine.
H-Cq., Hydroxychloroquine.

TABLE 2. Clinical and serological features in patients with chronic cutaneous LE

Patient No.	Sex	Age at onset (years)	Disease duration (years)	Therapy (mg)	ANA
1	F	44	13	Topical steroid	+
2	F	16	43	Topical steriod	–
3	F	40	3	Mepacrine 200	–
4	F	11	20	Topical steroid	–
5	F	68	9	Mepacrine 100	–
6	F	28	2	Topical steroid	–
7	F	24	10	Topical steroid	–
8	F	46	16	Topical steroid	–
9	F	41	5	Topical steroid	–
10	F	30	37	Topical steroid	+
11	F	28	37	Topical steroid	+
12	F	35	5	Topical steroid	–
13	F	30	24	Hydroxychloroquine 400	–
14	F	51	12	Mepacrine 50	–
15	F	32	22	Mepacrine 100	–
16	F	40	18	None	–
17	F	28	3	Inactive	–
18	F	40	6	Topical steroid	–
19	F	27	4	Topical steroid	–
20	M	31	6	Topical steroid	–
21	M	14	54	Topical steroid	–
22	M	44	2	Topical steroid	–
23	M	73	5	None	–
24	M	14	14	Topical steroid	–
25	M	38	6	None	–
26	M	35	9	Topical steroid	–
27	M	53	12	Topical steroid	–
28	M	27	4	Inactive	ND
29	M	44	5	Topical steroid	–
30	M	37	3	Topical steroid	ND
31	M	37	7	Topical steroid	–
32	M	30	11	Topical steroid	–

+, Positive or involved.
–, Negative or not involved.
ND, Not done.

Similar conclusions have been drawn from studies of the LBT in oral mucosa in LE,[6] but in these studies the majority of biopsies have been taken from keratinized palatal mucosa.[4–6] Ulceration of non-keratinized mucosa occurs in SLE and chronic plaques may develop on non-keratinized buccal mucosa in CCLE. Although it has been suggested that non-keratinized mucosa is a less sensitive site for the detection of immunoreactants in LE,[9] the prevalence of immunoreactants in uninvolved non-keratinized oral mucosa in patients with SLE or CCLE has not been established. Ocular involvement may occur in SLE as well as CCLE[10,11] but the immunopathology of conjunctiva has not been studied in LE.

We have examined the prevalence and clinical significance of subepithelial immunoglobulin and complement deposition in uninvolved sun-protected forearm skin, uninvolved sun-protected non-keratinized lip mucosa and uninvolved sun-protected bulbar conjunctiva of patients with SLE or CCLE.

METHODS

Patients

Systemic LE. Twenty patients with SLE (two male; 18 female) were included in the study and the clinical details are shown in Table 1. The patients fulfilled the 1982 revised criteria of the American Rheumatism Association for the diagnosis of SLE.[12] The disease duration ranged from 9 months to 31 years (mean 11·6 years) and the age of onset from 18 to 54 years (mean 34·7 years). Sixteen patients with well controlled disease were on treatment with immunosuppressive drugs (prednisolone: average 9 mg/day, range 2·5–30 mg/day; azathioprine 50–150 mg/day; mepacrine 50 mg/day; hydroxychloroquine 200–400 mg/day); and one patient with disease of recent onset was included in the study before systemic treatment commenced. One patient with mild disease and one patient with inactive disease did not require systemic therapy.

TABLE 3. Clinical and immunoflorescence findings in patients with systemic LE

	Clinical features				Direct immunofluorescence of uninvolved, non-exposed tissue		
Patient No.	Chronic cutaneous (discoid) LE	Nasal septum	Oral mucosa	Episcleritis	Skin	Lip mucosa	Conjunctiva
1	+	–	–	–	–	–	IgG
2	–	–	–	–	–	–	IgG
3	+	–	–	–	–	IgM	–
4	–	+	–	–	IgM,C1q	–	–
5	+	–	+	–	–	–	ND
6	–	–	–	–	–	–	ND
7	–	–	–	+	IgA,IgM,C1q	IgG,IgA,IgM,C1q	IgG
8	–	–	–	–	ND	ND	–
9	–	–	–	–	–	–	ND
10	–	–	–	+	–	–	ND
11	–	–	–	–	–	–	ND
12	–	–	–	–	–	–	–
13	–	–	–	–	C1q	–	ND
14	+	–	–	–	–	–	IgM,IgG,C3
15	+	–	–	–	C3	–	ND
16	–	–	–	+	–	–	–
17	–	–	–	+	–	–	–
18	–	–	–	–	–	–	–
19	–	–	–	–	IgM	IgM	ND
20	+	–	–	+	IgG,C1q	IgG,C1q	C1q

+, Involved.
–, Negative or not involved.
ND, Not done.

Chronic cutaneous LE. Thirty-two patients with CCLE (13 males, 19 females) were included in the study and the clinical details are shown in Table 2. Eighteen patients had localized CCLE (disease confined to the head and neck) and 14 had generalized CCLE (lesions present below the chin). The diagnosis in these cases had been confirmed histologically. The disease duration ranged from 2 to 54 years (mean 12·8 years) and the age of onset ranged from 11 to 68 years (mean 35·8 years). At the time of the study the majority of patients were using topical corticosteroids and sunscreens. Five patients were taking antimalarials, either mepacrine 50–200 mg a day or hydroxychloroquine 400 mg a day. Two patients who had no lesions at the time of the study and three patients with mild disease did not require treatment.

Controls. Bulbar conjunctival biopsies were obtained from 50 patients (24 male, 26 female; age range 1–88 years; mean age 63 years) undergoing ocular surgery for conditions unrelated to connective tissue diseases, such as cataract, squint or retinal detachment. Patients with known primary conjunctival disease were not included.

Clinical methods

A detailed history was taken, particularly of symptoms involving the eyes, nose, lips, mouth and perineum. Mucosal surfaces and perineum were examined. Patients with oral signs were assessed by an oral surgeon and when consent was obtained the diagnosis was confirmed by an oral biopsy. Patients were referred for full assessment by an ophthalmologist (PAF) and the anterior ocular surface was examined using a slit lamp.

Biopsies

Informed consent was obtained from the patients. Single 3 mm punch biopsies were taken from uninvolved, non sun-exposed, forearm skin, uninvolved non sun-exposed lower lip mucosa and uninvolved non sun-exposed bulbar conjunctiva. There were 19 forearm biopsies, 19 lip mucosal biopsies and 12 conjunctival biopsies from patients with SLE. There were 32 forearm biopsies, 29 lip mucosal biopsies and 20 conjunctival biopsies from patients with CCLE. All biopsies were embedded in OCT medium and snap frozen in liquid nitrogen.

Cryostat sections of the biopsies were examined for the presence of IgA, IgG, IgM and C3, C4 and C1q using standard direct immunofluorescence techniques. A positive lupus band test (LBT) was defined as linear deposition of an immunoreactant at the basement membrane zone (BMZ).

Statistical methods

The distribution of positive lupus band tests in skin, lip and conjunctiva in patients with CCLE and SLE were compared using the χ^2 test corrected for small numbers.

RESULTS

Lupus band test [LBT]

SLE. The results are shown in Table 3. Linear deposition of an immunoreactant at the BMZ was detected in 32% (6/19) of skin biopsies; 21% (4/19) of lip mucosal biopsies and 42% (5/12) of conjunctival biopsies. There was no significant difference in the sensitivity of the test at different sites in SLE and no correlation between a positive LBT in skin, lip or conjunctiva and clinical mucosal involvement.

TABLE 4. Clinical and immunofluorescent findings in patients with chronic cutaneous LE

	Clinical features					Direct immunofluorescence of uninvolved, non-exposed tissue		
	CCLE							
Patient No.	Localized	Generalized	Nasal septum	Oral mucosa	Conjunctiva	Skin	Lip mucosa	Conjunctiva
1	+	–	+	–	–	–	–	–
2	–	+	–	–	–	–	–	ND
3	–	+	–	–	–	–	–	IgG
4	+	–	–	–	–	–	–	–
5	–	+	–	–	–	IgM	–	–
6	–	+	–	–	–	–	–	IgG
7	+	–	–	–	–	–	ND	ND
8	–	+	–	–	–	–	–	ND
9	–	+	–	–	–	–	IgM	–
10	–	+	–	–	–	–	–	–
11	+	–	+	–	+	–	–	IgG
12	+	–	–	–	–	–	–	IgG
13	+	–	+	–	–	–	–	–
14	+	–	–	–	–	–	–	IgG
15	–	+	–	+	–	–	–	–
16	–	+	–	–	–	–	–	ND
17	–	+	–	–	–	–	–	ND
18	+	–	–	+	–	–	–	ND
19	+	–	–	–	–	–	–	IgG
20	+	–	–	–	–	–	–	ND
21	–	+	–	–	+	–	–	IgG,IgA,C_4
22	+	–	–	–	–	–	–	ND
23	–	+	–	–	–	–	–	IgG
24	–	+	+	–	–	–	–	ND
25	+	–	–	–	–	–	–	ND
26	+	–	+	+	–	–	–	IgG
27	–	+	–	–	–	–	ND	ND
28	+	–	–	–	–	–	–	–
29	+	–	–	–	–	–	–	–
30	+	–	–	–	–	–	ND	ND
31	+	–	–	+	–	–	–	–
32	+	–	–	–	–	–	–	IgG

+, Involved.
–, Negative or not involved.
ND, Not done.

CCLE. The results are shown in Table 4. Linear deposition of an immunoreactant at the BMZ was found in 3% (1/32) of skin biopsies; 3% (1/29) of lip mucosal biopsies and 50% (10/20) of conjunctival biopsies (Fig. 1). Thus, in CCLE there was a significantly greater chance of finding a positive LBT in conjunctiva than in skin or in lip [$P < 0{\cdot}001$, χ^2 test]. There was no correlation between a positive LBT in skin, lip or conjunctiva and the presence of clinical mucosal involvement.

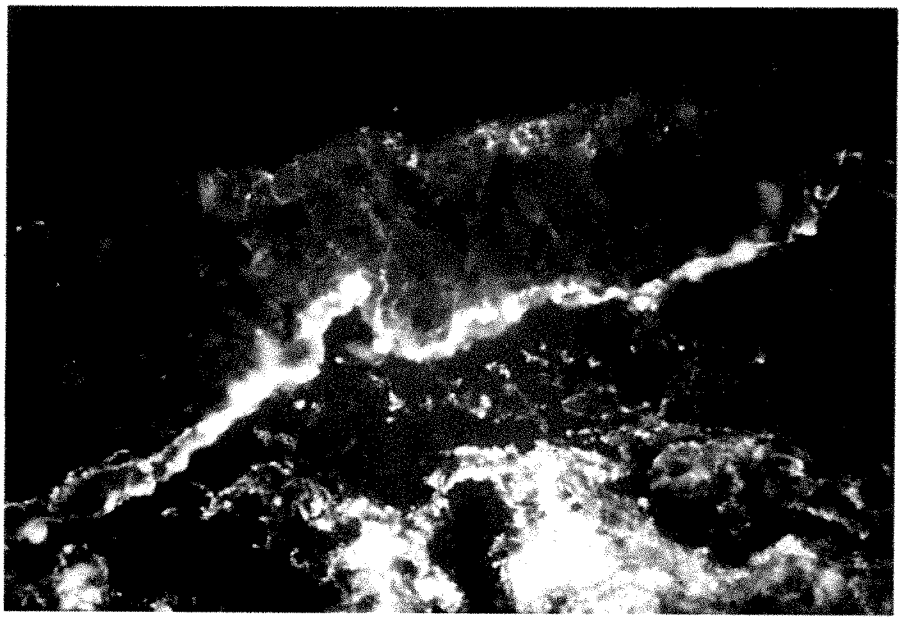

FIGURE 1. Conjunctival biopsy from a patient with CCLE showing linear, granular deposition of IgG at the basement membrane zone.

Immunoreactants. The distribution of immunoreactants is shown in Fig 2. In both SLE and CCLE the immunoreactant found in conjunctiva was almost exclusively IgG. C1q was deposited in the BMZ in the conjunctiva in one patient with SLE. In contrast, in the biopsies from skin and lip, complement components and IgM were detected more often than IgG.

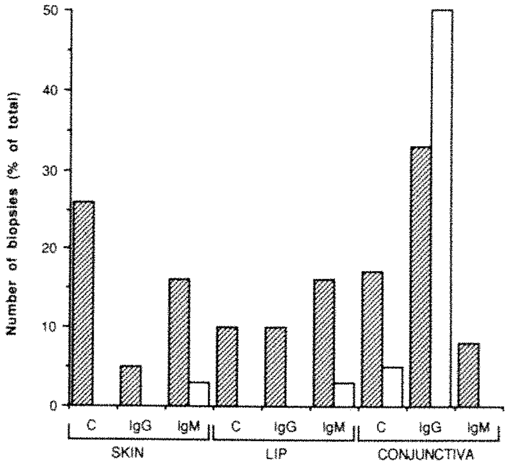

FIGURE 2. Distribution of immunoreactants in positive lupus band tests in uninvolved, sun-protected skin, lip and conjunctiva. In CCLE, there was a much greater chance of finding a positive LBT in conjunctiva than in skin or lip and in both SLE and CCLE the immunoreactant detected in conjunctiva was predominantly IgG. ▨, SLE; □, CCLE, C = C1q, C3 or C4.

Controls. A positive result was found in five of the 50 control conjunctival biopsies. One of these patients had a renal transplant and was on immunosuppressive therapy, and this result was excluded from the analysis and another had thyroid eye disease. IgG was present at the BMZ in 6% (3/49) of the conjunctival biopsies and IgM was the sole reactant in one biopsy.

DISCUSSION

Immunoreactants were present in uninvolved skin in 32% (6/19) of SLE patients. More than one immunoreactant was present at the BMZ in half the positive tests and one patient, with active disease of recent onset, had three immunoreactants in the skin. According to previous studies the specificity and predictive value of the LBT increases with the number of immunoreactants detected,[13] but the origin and significance of immune deposits in skin in LE is uncertain.

Cutaneous immunoglobulin deposits in SLE possess anti-nuclear antibody activity[14,15] and cross reactive anti-DNA antibody idiotypes have been detected on tissue-bound immunoglobulins from skin biopsies in both SLE and CCLE.[16] Subepidermal accumulation of immunoglobulin in uninvolved skin in SLE may depend upon the presence of serum anti-nDNA antibody.[2] Although there is no correlation between the presence of subepidermal immunoreactants and active skin disease, neurological involvement, arthritis, serositis or vasculitis, there does seem to be a significant positive correlation between the presence of linear BMZ immunoreactants in uninvolved sun-protected skin and clinical renal disease,[3] so that the result of a LBT may have prognostic significance. SLE patients with a positive test composed solely or in part of IgG have a greater prevalence of anti-DNA antibodies and renal disease than other patients.[3,13] Only one of our patients had IgG in the BMZ of the skin biopsy. The low number of positives in our study reflects the fact that most patients were on immunosuppressive therapy and had well-controlled disease with low titres of circulating anti-nDNA.

A band composed solely of IgM was found in an untreated patient with long standing SLE, normal renal function and anti-Ro antibodies. Untreated SLE patients with an IgM band usually have chronic low grade activity and often have anti-nRNP, Ro and La antibodies.[3] IgM was also detected in the BMZ in 3% (1/32) of patients with CCLE, but deposits of IgM in the BMZ of clinically normal skin have been reported in healthy individuals as well as a variety of dermatoses and are not specific for LE.[17,18] Granular deposits of IgM have been detected in the BMZ of normal skin in more than 20% of a fit control population[19] and the predictive value of an IgM band for SLE is low.[13]

Our results for lip mucosa are similar to those of previous studies using keratinized oral mucosa and demonstrate that the LBT in uninvolved non-keratinized oral mucosa may be positive in SLE, but is usually negative in CCLE.[4,6] As in the skin, the overall positivity was low, but the patient with the most active systemic disease had four immunoreactants in the lip mucosal biopsy and three immunoreactants in the skin biopsy.

A band of immunoreactants may be detected at the basement membrane zone in cutaneous and mucosal lesons in LE.[3,4,20] It is unclear if immune deposits have a pathogenic role but since immunoreactants are present in uninvolved skin and mucosa, it is unlikely that they play a primary role in the pathogenesis of cutaneous or mucosal disease.[3,21]

There have been no previous studies of the immunopathology of conjunctiva in LE. Our results showed a high positivity rate of the LBT in conjunctiva particularly in patients with CCLE. The test was positive in 50% (10/20) of the biopsies in CCLE and 42% (5/12) of the biopsies in SLE. We also found a predominance of IgG in the positive tests: IgG was present in all but one biopsy but was the only immunoreactant in 90% (9/10) of positive CCLE biopsies and 60% (3/5) of positive SLE biopsies.

We were unable to correlate the presence of a positive conjunctival LBT with local or systemic clinical features. Of the SLE patients, one with IgG and one with CIq had episcleritis, but two patients had a past history of episcleritis without deposits at the time of biopsy. Two patients with CCLE had pronounced eyelid involvement and conjunctival scarring with symblepharon. The test was performed on uninvolved bulbar conjunctiva and was positive in both these patients. None of the other patients with positive results had local involvement. Once the disease is inactive, IgM may persist longer than other immunoreactants in the BMZ of skin and oral mucosa,[3,6] but our results suggest that in conjunctiva IgG is either preferentially deposited or cleared more slowly than other immunoreactants.

In skin, the lupus band depends upon the presence of serum anti-nDNA and is composed of nDNA: anti-nDNA complexes, but a positive conjunctival LBT occurred in patients with CCLE. These patients did not have serum anti-DNA antibodies and it seems unlikely that the deposits in conjunctiva possess anti-nDNA activity. There is evidence that some patients with CCLE have polyclonal B-cell activation,[22,23] and immunoglobulin deposition in the conjunctiva in CCLE may reflect a non-specific increase in circulating immunoglobulin secreting cells. It is not clear why patients with CCLE do not suffer from the clinical consequences of excessive autoantibody production or immune complex formation. It is interesting that one of our control patients with linear IgG in the conjunctiva had autoimmune thyroid eye disease while another was a renal transplant patient, and both these 'normal' control patients may have had some degree of B-cell activation. Direct immunofluorescence studies of conjunctiva, lower lip mucosa and skin in bullous and cicatricial pemphigoid by our group also demonstrated that the frequency of positive immunofluorescence was higher in the conjunctiva than in the lip mucosa or skin.[24]

In conclusion, this study has demonstrated firstly that the lupus band test in SLE may be positive not only in uninvolved sun-protected forearm skin but also in uninvolved sun-protected non-keratinized lip mucosa. Secondly, in both SLE and CCLE the test may be positive in 40–50% of biopsies from uninvolved sun-protected bulbar conjunctival mucosa and in CCLE the test may be positive in conjunctiva when skin and lip are negative. Presumably local factors influence the deposition and persistence of conjunctival subepithelial immunoreactants. Subepithelial conjunctival immunoreactants may be present in other autoimmune diseases and in some groups of patients, conjunctiva may be the preferred site of biopsy for a diagnostic immunofluorescence test. This possibility deserves further investigation.

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Treatment of dermatophyte infection of the finger- and toe-nails with terbinafine (SF 86-327, Lamisil), an orally active fungicidal agent

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SUMMARY

We report on the use of a new orally active fungicidal agent, terbinafine (SF 86-327, Lamisil) in the treatment of patients with dermatophyte onychomycosis. Twenty patients with toe-nail, and 10 with finger-nail infection received 250 mg of terbinafine daily: finger-nail infections were treated for 6 months and toe-nail infections for 12 months. All 24 patients who completed the course of therapy achieved mycological cure, as did two subjects who dropped out of the trial. All but two patients had clinically normal nails at the end of the study period. The mean time for mycological cure was 12·5 weeks for finger-nail infection, and 24 weeks for toe-nail infections. The time for a clinical cure with normal nails was 20·5 weeks for finger-nail infection, and 44 weeks for toe-nail infection. An exacerbation of pre-existing dyspepsia occurred in three of the six patients who did not complete the trial but there were no other significant adverse reactions.

Dermatophyte infection of the nails with associated tinea pedis is a common problem and standard treatment consists of oral therapy with griseofulvin, often for as long as 18 months. The cure rate with griseofulvin is variable, and may be as low as 20% for toe-nail and 40% for finger-nail infection[1] with relapse of around 20% at 1 year after stopping therapy.[2] There are side-effects from using this anti-fungal drug. Also, long-term treatment with oral ketoconazole is in relative disrepute after reports of fatal liver damage.[3] The new nail penetrating tioconazole lotion, although found to be promising[4] is possibly only to be used as an adjunct to oral therapy.

Allylamines, a new class of antifungal agents, have been reported to be effective in the treatment of fungal disorders.[5] Terbinafine (Sandoz Products Ltd.) is an orally active, synthetic antifungal of the allylamine class. It acts by inhibiting ergosterol biosynthesis and this produces

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sterol deficiency with intracellular accumulation of squalene, the inhibition occurring at the point of squalene epoxidation.[6] It is active *in vitro* against all dermatophytes causing onychomycosis as well as against many pathogenic yeasts. It is well absorbed when given orally, and rapidly metabolized to inactive metabolites which are excreted by both renal and biliary routes. Only minor gastrointestinal upsets have occurred from the use of this drug.

We report the use of the orally active fungicidal allylamine terbinafine (SF 86-327, Lamisil), in the treatment of onychomycosis due to dermatophyte fungi.

METHODS

Thirty patients aged over 18 with mycologically proven distal onychomycosis due to a dermatophyte were entered into the trial. All gave informed consent to the investigation which was approved by the local hospital ethical committee. Seven patients had received previous unsuccessful therapy with griseofulvin. Each patient received 250 mg of terbinafine daily, either as 125 mg b.d. or 250 mg at night in a double-blind, randomized parallel group trial comparing two dosage regimes of terbinafine. Patients with toe-nail infection were treated for 48 weeks and those with finger-nail infection for 24 weeks.

Full clinical and mycological assessment and routine haematological and biochemical tests were performed before entry into the trial. Only patients with positive mycological cultures were included in the trial. Those with abnormal laboratory screening tests, those who had received antifungal therapy within 3 months, those on immunosuppressant therapy, the oral contraceptive pill, or with a history of renal, liver or gastrointestinal disease or psoriasis were excluded at the outset.

Patients were assessed clinically and mycologically at 4-weekly intervals. The nail most severely involved was taken as a target for specific measurement with the extent of involvement marked by cutting a groove in the nail and marking it with ink according to the method of Zaias,[7] and the amount of uninvolved nail measured. At each clinic attendance this nail was measured.

Routine haematological and biochemical tests were repeated at each monthly review. Any patient developing symptoms or abnormal laboratory tests which were potentially due to their treatment was withdrawn from the trial. Photographs of the target nail were obtained at the beginning and end of treatment.

TABLE 1. Clinical and mycological details of 30 patients with dermatophyte onychomycosis

Age (years) mean (range)	47 (19–74)
Sex	20 male, 10 female
Site of infection	20 toe-nail, 10 finger-nail (2 both)
Duration of infection mean (range)	2·8 years (4 months–20 years)
Number of nails involved mean (range)	6 (1–15)
Infecting fungus	27 *Trichophyton rubrum*, 2 *Trichophyton mentagrophytes* 1 mixed infection with *T. rubrum* and *Epidermophyton floccosum*

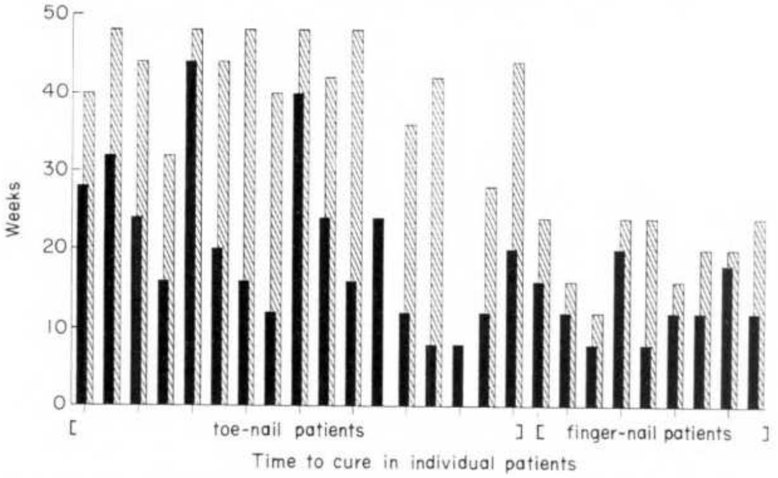

FIGURE 1. Time taken to achieve mycological and clinical cure in individual cases. ■, mycological cure; ▨, clinical cure.

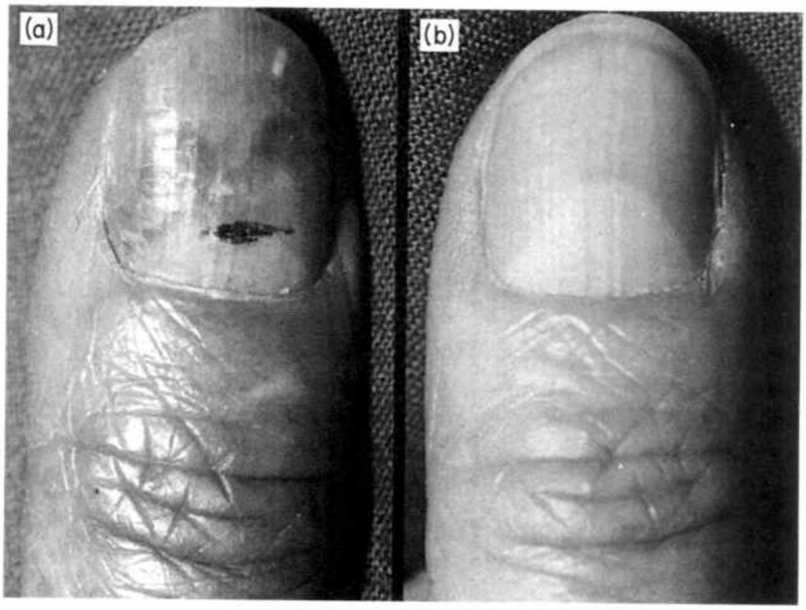

FIGURE 2. (a) Finger-nail with dermatophyte infection before treatment. (b) same finger-nail after 20 weeks treatment with terbinafine.

RESULTS

The clinical data of the patients enrolled in the study are given in Table 1. Six patients failed to complete the study. One decided that he did not wish to have his infection treated after all and three developed an exacerbation of pre-existing dyspepsia and withdrew after 4, 16 and 24 weeks respectively, the latter two having achieved a mycological cure. Two patients defaulted from follow-up after achieving both a mycological and a clinical cure.

Figure 1 shows the time to mycological and clinical cure. Mycological cure was assumed when two successive nail samples were negative for fungus both on microscopy and on culture. Clinical cure was taken as the time when the target nail was clinically normal (Figs 2a and b). Since the target nail was always the worst affected, clinical cure meant that all the previously affected nails were normal. Two patients who completed 12 months of treatment had residual nail abnormalities. One developed psoriasis with nail involvement and the second had a small area of discoloured nail that was still abnormal 6 months later, though mycological examination remained negative. The median time to mycological cure was 12·5 weeks in those with finger-nail infection, and 24 weeks in those with toe-nail infection. The time to both clinical and mycological cure depends on the growth rate of nail, as well as on the antifungal effect of the drug, but was 20·5 weeks for finger-nail infection and 44 weeks in toe-nail infection.

Mild dyspepsia occurred in five patients, without having to discontinue therapy, but no other symptoms attributable to the drug occurred. There were no significant changes in the haematological or biochemical tests. There were no differences between the two treatment regimes in any of the variables assessed.

Follow-up is so far incomplete, but of the 24 patients who completed the study, 20 remain mycologically disease-free after 12 months off treatment. Three are disease free after 6 months and one was clear at 3 months but has been lost to follow-up. Full analysis of the two dosage regimes is not yet available, however, since the success rate of treatment is high, and the incidence of significant side-effects low, it is unlikely that there will be any important difference.

DISCUSSION

This study shows that terbinafine is an effective treatment for dermatophytosis of the nails. The mycological cure rate was 100% in those patients who completed their course of treatment, and the clinical cure rate was 92% according to the strict definition used here, i.e. normal nails at the end of treatment. In practical terms, clinical cure was also 100% since the nail abnormality that persisted in two patients was not due to a persisting fungal infection. The drug also seems to work rapidly, probably due to the fungicidal action of the drug, with a median time to mycological cure of only 12·5 weeks in those patients with finger-nail infection, and approximately twice that in toe-nail infections; this difference probably reflects the faster growth rate of finger-nails[8] and possibly an enhanced incorporation of the drug in finger-nails, although this remains speculative.

The rapid rate of mycological cure compared to that achieved with griseofulvin[1] raises the possibility that much shorter durations of treatment might be equally successful. Although follow-up of these patients is so far incomplete, all patients who have been followed up for a year (20 out of 26) remain disease free.

The other important aspect of a new drug is that of safety. Terbinafine appears to be well tolerated. The only side-effect experienced by any of our subjects was dyspepsia, the occurrence of which has been previously noted. Three patients out of 30 had to discontinue therapy because of dyspepsia but this occurred only in subjects with a previous history of this condition and two had similar problems after subsequent treatment with griseofulvin. Caution is obviously required in patients with a dyspeptic history with both terbinafine and griseofulvin. There was no evidence of haematological or biochemical disturbance due to the drug in any of the patients treated, even after 12 months treatment. This is encouraging in view of the toxicity noted with other oral antifungal drugs.

Our preliminary data suggest that terbinafine may be a most useful addition to the limited number of antifungal agents available for the treatment of dermatophytosis of the nails. Further data about relapse rates and about longer term safety are currently being collected.

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Sulphamethoxypyridazine for dermatitis herpetiformis, linear IgA disease and cicatricial pemphigoid

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SUMMARY

One-hundred and sixty-eight cases of dermatitis herpetiformis were reviewed to compare the clinical response to and incidence of side-effects from dapsone and sulphamethoxypyridazine. Thirty-seven received sulphamethoxypyridazine (0·25–1·5 g/day) as a single agent therapy at some stage during their care and 161 had dapsone only (50–450 mg/day). Thirty of these patients received both drugs, but at different times. Both were highly effective in controlling the skin disease in 97% of patients on dapsone and 89% on sulphamethoxypyridazine. While 36 (22%) of dapsone-treated subjects had intolerable side effects warranting a change in therapy, this occurred in only five (13·5%) of those treated with sulphamethoxypyridazine. Sulphamethoxypyridazine was also effective as a single agent in three patients with linear IgA disease who had suffered adverse effects from dapsone, and in 10 out of 15 patients with oral and cutaneous lesions of cicatricial pemphigoid.

Dermatitis herpetiformis (DH), linear IgA disease (LAD) and cicatricial pemphigoid (CP) are disorders characterized by inflammatory subepidermal bullae. Dapsone is the main drug used in the treatment of DH, but in some patients intolerable side-effects occur and alternative therapies have to be used. Sulphamethoxypyridazine (SMXP) is an effective alternative drug in the therapy of DH, LAD and CP.

METHODS

The data was collected from patients attending a bullous disease clinic at intervals of not greater than 12 months. The diagnosis of DH was based on the clinical appearance of the rash together with demonstration by direct immunofluorescence of IgA deposits in the papillary dermis of

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uninvolved skin. The rash in these patients was initially controlled by drug therapy, and a strict gluten-free diet instituted. In each patient the minimum dose of drug required to control the rash was noted, together with any side-effects. LAD was identified by the presence of subepidermal blisters with a homogeneous and linear deposition of IgA at the dermo–epidermal junction of uninvolved skin as seen using direct immunofluorescence. CP was identified as a blistering disease of the mucosa and skin with scarring of the affected areas, particularly the conjunctiva.

One-hundred and sixty-eight patients with DH, 13 with LAD and 15 with CP disease were included in the study.

RESULTS

Dermatitis herpetiformis

Dapsone-treated group. One-hundred and fifty-six out of 161 patients (97%) treated with dapsone (mean dosage 100 mg/day, range 50 mg/week–450 mg/day) had a completely satisfactory therapeutic response. An improvement usually occurred within 48 h of the start of treatment. Some had minor complaints such as nausea or lethargy, but not sufficient to warrant a change of therapy. The adverse effects sufficient to warrant change in therapy are listed in Table 1 and in 24 subjects with intolerable side-effects therapy was changed to SMXP.

Sulphamethoxypyridazine (SMXP)-treated group. Thirty-seven patients received SMXP (22 male, 15 female; mean age 49 years, range 14–73 years). This was given in the form of 500 mg tablets in a daily dosage that ranged from 0·25–1·5 g/day (mean 740 mg/day). The control of symptoms and rash was satisfactory in all apart from four patients. Twenty-eight were treated with SMXP either because of poor control or marked side-effects while on dapsone or sulphapyridine. Elderly patients were considered to be less tolerant of the dapsone, especially with regard to the side-effect of this drug in inducing haemolysis. In these patients SMXP was the drug of first choice. The main adverse effects are listed in Table 1. Five patients (13·5%) suffered from significant side-effects and the mean dose in the group with adverse reactions was 820 mg/day (range 500–1000 mg/day). The mean age of the group with adverse reactions was 58 years (range 44–72 years), as compared with a mean age of 47 years (range 14–73) in those free from side-effects.

Adverse effects with SMXP. A 50-year-old woman was treated initially with SMXP, 500 mg/day, which completely controlled the rash within a week. At this stage she developed a generalized maculopapular rash which cleared on withdrawal of the drug. Therapy was changed to dapsone 50 mg/day with a gluten-free diet and after 16 months she was completely controlled on a gluten-free diet alone.

A 73-year-old man was started on SMXP, 500 mg/day and after 7 months the blood count dropped from Hb 13·0 g/dl, white cell count (WCC) $8{\cdot}7 \times 10^9$/l, to Hb 11·7 g/dl, WCC $3{\cdot}7 \times 10^9$/l. On stopping the SMXP tablets his Hb returned to normal (12·5 g/dl) with a WCC of $6{\cdot}9 \times 10^9$.

A 44-year-old woman who had been taking SMXP 0·5 g/day for 6 months complained of feeling unsteady on her feet and experiencing difficulty in co-ordination. Neurological examination revealed no obvious abnormalities, but her symptoms disappeared on stopping the drug. A 50-year-old man complained of similar unsteadiness which also disappeared on stopping the drug. Subsequent investigations showed that this patient had impaired renal function.

TABLE 1. Adverse effects of dapsone and sulphamethoxypyridazine (warranting change of therapy)

	Dapsone group	Sulphamethoxypyridazine group
Dermatitis herpetiformis		
No. treated	161	37
No. patients with side effects	36 (22%)	5 (13·5%)
Mean dosage (mg/day)	120	820
Range dosage (mg/day)	50–200	500–2000
Anaemia	16	1 (with leukopenia)
Headache	3	—
Malaise, lethargy	5	1
Heart failure	1	—
Depression	1	—
Renal papillary necrosis	1	—
Unsteadiness	—	2
Neuropathy	5	—
Thrombocytopenia	2	—
Drug-induced rash	—	1
Dyspnoea	1	—
Nausea	3	—
Angina	1	—
Linear IgA disease		
No. treated	11	3
Headache and nausea	1	—
Haemolysis	1	—
Angina	1	—
Motor neuropathy	1	—
Cicatricial pemphigoid		
No. treated	4	13
Pneumonitis	—	1

A 72-year-old man was commenced on SMXP, 500 mg/day and this rapidly controlled the rash, but a month later he noted that he was becoming increasingly tired and lethargic. This resolved on stopping the SMXP.

Linear IgA disease

Thirteen patients were studied (6 male, 7 female). Six patients were well controlled with dapsone (50–150 mg/day) but after various adverse effects three were changed to SMXP (1000 mg/day) with good control. One patient on 100 mg/day of dapsone developed a motor neuropathy and was treated with SMXP and sulphapyridine. Two patients had combination therapy with dapsone and SMXP. No adverse effects from SMXP were reported in this group.

Cicatricial pemphigoid

Fifteen patients (4 male, 11 female) were studied. The following refers only to the response to treatment of the cutaneous and oral lesions. Nine responded well to SMXP (dose range 500–1500 mg/day). One patient was not controlled on 100 mg/day of dapsone but was controlled on SMXP 500 mg daily. One patient was not controlled with either dapsone or SMXP, but eventually on sulphapyridine. One patient required a combination of SMXP and dapsone.

Three did not respond to any drug, nor to combinations of SMXP with dapsone or sulphapyridine.

There were no adverse effects with dapsone. A 58-year-old female who had been on 1 g/day of SMXP for about 1 year developed increasing breathlessness with a reduced chest expansion, and on radiography had nodular infiltrative changes in both lungs. A diagnosis of alveolitis was ascribed to SMXP. She was commenced on prednisolone 30 mg a day and the SMXP was stopped with resolution of the lung changes.

DISCUSSION

This study demonstrates that SMXP is an effective alternative to dapsone in the treatment of DH, LAD and CP subepidermal bullous dermatoses.

In 1958 Jefferson reported 12 cases of dermatitis herpetiformis successfully treated with SMXP. The response was satisfactory in all cases and adverse reactions were almost entirely absent.[1] Of 19 DH patients treated with SMXP[2] it was found to be difficult to predict maintenance dose or stable blood levels per unit dose, although the control of DH was readily achieved using this drug. These findings were subsequently confirmed.[3]

The incidence of adverse side-effects associated with the use of SMXP was estimated in one report as 6%[4] and the frequency of side-effects in another as 6·6% on just 0·5 g/day compared to 15% for the group as a whole,[5] suggesting that the risk of adverse reaction is related to the dose of the drug.

In a group of 110 patients treated with SMXP, 10% had mild subjective symptoms but no serious side-effects.[6] Adverse reactions reported in the past have consisted of the common side-effects documented with other sulphonamides.[7] Perry and Winkelmann[2] found adverse reactions in 19 cases of DH and nine other complaints treated with SMXP. These included a 'toxic' reaction with pyrexia, arthralgia and albuminuria, an erythematous maculopapular rash, haemolytic anaemia, a fixed drug reaction and photosensitivity. Indeed most known adverse effects of sulphonamide drugs have been reported with the use of SMXP. In our patients we found no cases with non-reversible side-effects, and the low incidence of adverse reactions (13·5%) with SMXP compared favourably with dapsone. However, in one patient alveolitis developed and physicians prescribing SMXP should be aware of this possible serious side-effect. SMXP is a suitable alternative and could be the drug of choice in those patients who might not tolerate dapsone.

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A comparative trial of Clinitar* versus hydrocortisone cream in the treatment of atopic eczema

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SUMMARY

Thirty patients with mild to moderate bilateral atopic eczema were treated on one side of the body with a new purified coal tar cream (Clinitar) and on the other side with 1% hydrocortisone cream, for 4 weeks. The degree of infiltration of the skin erythema, lichenification, excoriations and dryness were assessed. Both treatments were equally effective in reducing symptoms and there was also no difference in patient preference and cosmetic acceptability.

For many years tar treatments have been considered an effective treatment for atopic dermatitis.[1,2] Tar is an antipruritic and has been shown to have an antimitotic effect,[3–5] although the precise mechanism of action is not fully understood. Since their introduction, corticosteroids have been more acceptable and easier to apply without the disadvantageous smell of tar preparations. Recently, improved tar preparations have been available and Stantar (pHarma medica a–s), a special extract of crude coal tar in which the heaviest fractions of the tar are filtered off, is more cosmetically acceptable. This trial compared the effectiveness and acceptability of Clinitar cream containing 1% Stantar with 1% hydrocortisone cream.

METHODS

Thirty patients suffering from mild to moderate bilateral atopic eczema were entered into the trial. There were 11 males and 19 females with a mean age of 11·8 years. The patients were treated twice a day with Clinitar cream on one side of the body and with 1% hydrocortisone cream on the other side over a period of 4 weeks. The treatments were randomly allocated to the left and right sides of the body. Prior to commencing treatment, each patient was assessed on both sides of the body using a 4 point scale for infiltration of the skin, erythema, lichenification, excoriations and dryness: 0, not present; 1, slightly affected; 2, moderately affected; 3, severely affected. Each patient was reassessed after 2 and 4 weeks of treatment, a note being made of any

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* Trade mark.

side-effects. After 4 weeks of treatment, both doctor and patient compared the two sides of the body for the overall therapeutic response, and the patient compared the cosmetic aspects of the two treatments.

During treatment patients were not allowed to take any other medication, either oral or topical, and bath oils and emollients were prohibited.

Statistical evaluation was performed by an independent statistician. The data for each treatment were analysed for linear regression of symptom score, and the treatments were compared using McNemer's test, by assessing the intra-individual magnitude of symptom response to both treatments between assessments.

RESULTS

All the patients completed the study. Mean scores for each symptom are presented in Table 1. There was a linear reduction of scores during treatment with both Clinitar and hydrocortisone cream. This applied to infiltration (Fig. 1, $P > 0{\cdot}001$), erythema (Fig. 2, $P > 0{\cdot}001$), lichenification (Fig. 3, $P > 0{\cdot}001$), excoriation ($P > 0{\cdot}01$) and dryness ($P > 0{\cdot}001$). There was no significant difference between the two treatments.

Six patients had minor adverse effects during the treatment period, which were itching and soreness. Of these one was with hydrocortisone and five were on the side treated with Clinitar.

The patients were asked to state if they preferred one of the treatments with regard to either effectiveness or cosmetic acceptability. There was no statistical difference between the two treatments and most had no preference.

DISCUSSION

Both treatments were effective in relieving the symptoms in the cases of mild to moderate atopic eczema, and no statistical difference was found between the treatments.

Atopic eczema is a chronic disease that requires long-term treatment and it is desirable that effective treatments with minimal side-effects are used, particularly when condition is only of mild or moderate severity. In the past tar preparations have suffered from poor cosmetic acceptability because of colour and smell, and these have influenced compliance. Clinitar is a more cosmetically acceptable form of coal tar preparation and provides an effective alternative to 1% hydrocortisone cream in the mild to moderate cases of atopic eczema.

TABLE 1. Clinical symptoms

	Mean symptom score					
	Clinitar			Hydrocortisone		
	Pretreatment	Week 2	Week 4	Pretreatment	Week 2	Week 4
Infiltration	1·30	0·77	0·33	1·27	0·67	0·30
Erythema	1·13	0·47	0·33	1·17	0·33	0·30
Lichenification	1·43	0·73	0·43	1·43	0·73	0·30
Scratch marks	0·77	0·43	0·23	0·77	0·30	0·17
Dryness	1·37	0·43	0·30	1·33	0·43	0·33

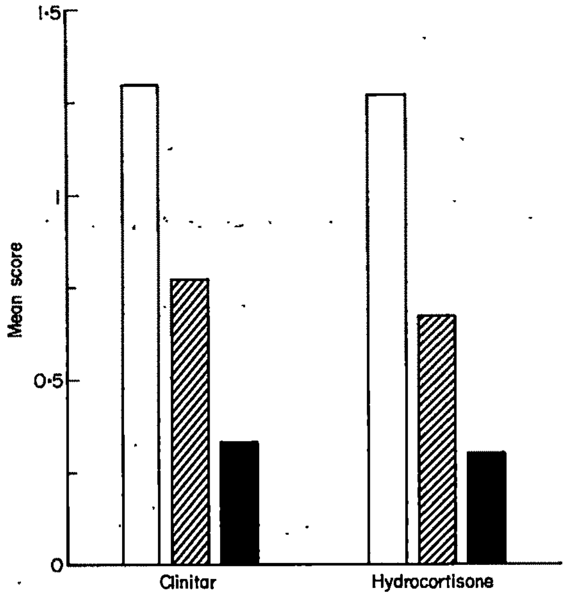

FIGURE 1. Mean scores for infiltration of the skin for Clinitar and hydrocortisone. □, Pretreatment; ▨, week 2; ■, week 4.

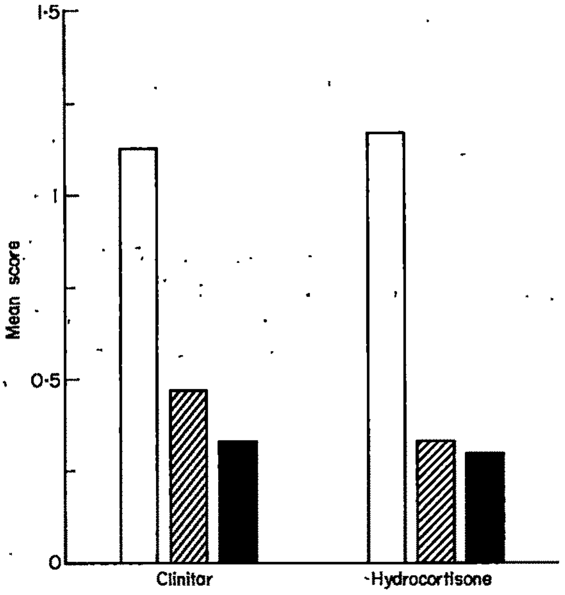

FIGURE 2. Mean erythema scores for Clinitar and hydrocortisone. For key see Figure 1.

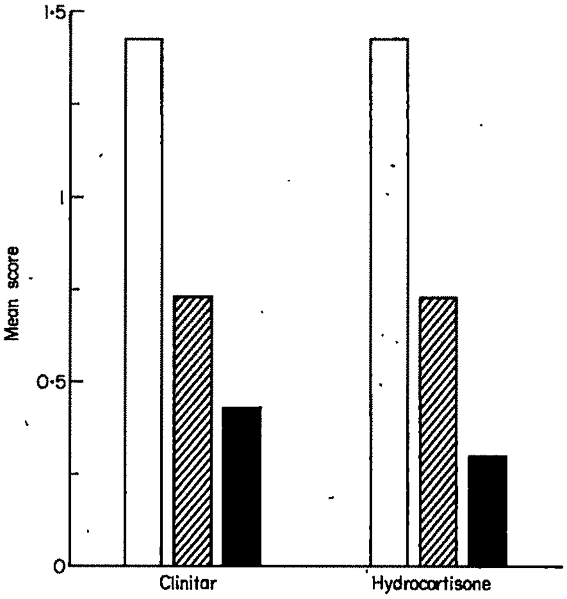

FIGURE 3. Mean lichenification scores for Clinitar and hydrocortisone. For key see Figure 1.

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Xeroderma pigmentosum and refractory anaemia in two first cousins

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SUMMARY

We report two first cousins with xeroderma pigmentosum who developed a refractory anaemia with excess of blasts which resulted in their deaths.

Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder with defective DNA repair. These patients frequently develop skin or eye malignancies but the incidence of internal neoplasms is not precisely known. A 10- to 20-fold increase was found in a retrospective survey of the literature[1] and there was a disproportionate increase of brain tumours and tumours of the oral cavity. Contrary to other DNA repair disorders such as ataxia telangiectasia, Bloom's syndrome and Fanconi's anaemia, haematological conditions have rarely been reported in association with XP. In a survey of 830 published cases, only two cases of XP were associated with leukaemia.[2] We report two first cousins with severe XP who developed in their mid-twenties a rapidly fatal myelodysplastic syndrome. Such an association has, to our knowledge, never been previously reported.

CASE REPORTS

Case 1

G.A. was an Algerian female born in March 1958 with consanguineous but healthy parents and diagnosed as having XP at the age of 5. She was the second of nine children and the seventh and eighth children of the family also suffered from XP. The seventh child died at the age of 8 from an unknown cause and the eighth had Down's syndrome.

She developed many actinic keratoses on the face and a basal cell carcinoma was removed from the forehead at the age of 19. At the age of 20, a corneal transplant was performed because of opacification and the patient received chloramphenicol eyedrops for a year following surgery.

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In January 1982 a benign multinodular goitre was removed and at that time no haematological abnormalities were noted. In November 1982, she was admitted for a pericarditis and concurrently had diffuse bleeding from the alimentary tract. Haematological tests showed a profound pancytopenia and 5% circulating blast cells (Table 1). A bone marrow biopsy showed increased cellularity and no myelofibrosis, but did show intense trilineage dyspoiesis. The percentage of blast cells was 10% with dysgranulopoiesis (nuclear abnormalities and hypogranular cells). Mature granulocytes were rare and had the same abnormalities. Active erythropoiesis was noted (25% erythroblasts) and there were nuclear and cytoplasmic abnormalities in these cells. Ringed sideroblasts were not found. The megakaryocytes were numerous but with various abnormalities. Karyotype analysis and complementation group determination were not performed. Blood transfusions and prednisolone 0·5 mg/kg per day did not produce any improvement (Table 1) and the patient died in January 1983 from profuse and uncontrollable haemorrhage. At that time the number of circulating blast cells had reached 12%.

Case 2

G.M., first cousin of G.A., was born in December 1959, the only child of healthy consanguineous parents. The diagnosis of XP was made at the age of 4. Five squamous cell carcinomas were removed from the face between 1973 and 1985 and a squamous cell carcinoma of the conjunctiva led to removal of the right eye in 1983. The patient was hospitalized in June 1986 because of a pyrexia and *Staphylococcus aureus* was found in blood cultures. He was successfully treated with methicillin 6 g/day and amikacin 600 mg/day. The haematological findings at that time showed a profound pancytopenia with 8% circulating blast cells (Table 1) and the bone marrow smear was hypercellular. There was a granulocytic hyperplasia with dysgranulopoiesis and blast cells, with Auer rods, represented 22% of the nucleated elements.

TABLE 1. Haematological findings in the two cases

	Case 1			Case 2		
	10 November 1982	6 December 1982	15 January 1983	6 June 1986	27 July 1986	25 August 1986
RBC (10^9/l)	2·4	3·12	0·65	2·52	1·91	2·1
Haemoglobin (g/l)	5	8·7	2·1	8	7	8
Haematocrit (%)	16·1	25·6	5·6	21	20	21
WBC (10^9/l)	0·3	3·2	2·3	2·07	4·7	4·7
Platelets (10^9/l)	28	19	04	42	17	13
Neutrophils (%)	50	20	31	51	32	25
Lymphocytes (%)	30	64	40	31	24	30
Monocytes (%)	07	08	04	—	02	03
Eosinophils (%)	03	01	05	03	19	10
Basophils (%)	—	—	01	02	03	05
Blast cells (%)	05	05	12	08	19	15
Myelocytes (%)	02	01	04	01	01	05
Metamyelocytes (%)	03	01	03	04	—	07

Promyelocytes showed important cytological anomalies with large primary granules. Several metamyelocytes and myelocytes appeared hypogranular or agranular. The erythroid precursors were quantitatively normal (30% erythroblast) but had features of dyserythropoiesis — multinucleated erythroblasts and punctate basophilia. Ring sideroblasts were observed but were below 10%. The megakaryocytes were numerous but small. Chromosomal analysis and complementation group determination were also not performed in this case.

Tests for immunological function were performed and delayed hypersensitivity skin testing with recall antigens: candida, trichophyton, phytohaemagglutin (PHA) gave a poor response, and lymphocyte transformation with PHA showed a significantly diminished response. There was a significant reduction in the proportion of circulating $CD3^+$ lymphocytes in comparison to normal controls and a decrease of $CD4^+$ cells (300/mm^3), whereas the $CD8^+$ cell subpopulation was normal (600/mm^3). The patient had four 7-day courses of intravenous low-dose cytosine arabinoside (0·7 mg/kg per day) but the pancytopenia persisted and the percentage of circulating blast cells gradually increased (Table 1). The patient died in September 1986.

DISCUSSION

Myelodysplastic syndromes are preleukaemic states in which a clonal abnormality of the haemopoietic stem cell leads to defective haemopoiesis with a reduction of peripheral blood cells and a cellular bone marrow. Five syndromes are now recognized:[3] refractory anaemia (RA), RA with ring sideroblasts, RA with excess of blasts (RAEB), chronic myelomonocytic leukaemia (CMML) and RAEB in transformation (RAEBt).

Our first case initially showed the characteristics of RAEB, but at the time of death the percentage of circulating blast cells had reached 12%, leading to the diagnosis of RAEBt (Table 2). From the beginning, case 2 met all the characteristics of RAEBt (Table 2). The prognosis of RAEBt is poor, often with a rapid developement of acute myeloid leukaemia.[4] As in both our cases, many of these patients either succumb to infections or die from severe haemorrhage before the onset of leukaemia. As expected, the survival time of our two patients was short and the mean survival time for RAEB and RAEBt from a previous study[4] was only 10 and 4·5 months from diagnosis.

TABLE 2. Criteria for the diagnosis of RAEB and RAEBt[3]

RAEB	RAEBt*
Peripheral blood	
Cytopenia of 2 or 3 cell lines	≥5% blast cells
<5% blast cells	Auer rods in blast cells
Bone marrow	
Hypercellularity	20–30% blast cells
5–20% blast cells	Auer rods in granulocyte precursors
Trilineage dyspoiesis	

* Idem RAEB plus any of the following.

One of the most striking features of our cases was the young age of onset of myelodysplasia. This usually occurs in patients over 50 years of age but an increasing number of young patients are now seen. They are mostly patients who have received prolonged treatment with radiotherapy or cytotoxic drugs, particularly alkylating agents.[5] None of these treatments were given to our patients before their myelodysplasia became apparent.

We are not aware of any other case of myelodysplasia occurring in patients with XP, and this association in our two cases may be of significance. XP is a disease in which there is a defect in DNA repair and in which cellular hypermutability has been shown to occur with several carcinogens. In addition to UV light, various chemical and viral carcinogens have been found to elicit abnormal responses with various cell types of XP.[6–8] *In vitro* data indicate that benzopyrenes, nitrosoureas and aflatoxins[7] increase chromosome breakage and that there is a high incidence of chromatid exchanges with an increase of mutations in XP cells. Data obtained from cytogenetic studies[9] and from glucose-6-phosphate dehydrogenase isoenzyme marker analyses[10] indicate that the clonal disorder responsible for myelodysplasia develops through successive mutations. With the improvement of survival in patients with XP there is a longer exposure to dietary, environmental, iatrogenic and viral carcinogens. The increase of somatic mutations from the action of the various carcinogens on DNA repair-deficient haemopoietic stem cells results in the development of myelodysplasia as seen in our patients. In the first case there was the application over several months of eyedrops that contained chloramphenicol, a chemical known to induce reversible myelodysplasia when given systemically.[11]

Also an immunological defect may contribute to the development of abnormal clones.[12] Previous studies have shown various immunological abnormalities in patients with XP,[13-14] which are a reduced response of lymphocytes to PHA, diminished delayed hypersensitivity skin reaction and reduced $CD4^+$ lymphocyte subpopulation. The degree of immunological impairment appears to be directly related to the severity of XP.[13] Immunological abnormalities were observed in our second case with diminished responses to recall antigens, defective lymphocyte transformation to PHA and reduced number of circulating $CD4^+$ cells. However, it was difficult to determine whether these defects were specifically linked to XP or secondary to the myelodysplasia.

These two cases of XP in their twenties developing myelodysplasia were related. Hereditary cases of myelodysplasia have been previously reported[15] and it is possible that in these two cousins it was the result of a hereditary anomaly. As with other DNA repair disorders, haematological conditions can also occur in XP and we await with interest other reports of this association.

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Symptomatic zinc deficiency in breast-fed premature infants

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SUMMARY

We report two breast-fed premature infants who developed transient symptomatic zinc deficiency with scaly erythema of cheeks and napkin area, 9–13 weeks after birth. Serum zinc concentrations were 3·6 and 4·8 μmol/l, and the lesions healed rapidly in response to oral zinc supplements. Both mothers had low breast-milk zinc levels (2·3 and 3·2 μmol/l at 21 and 15 weeks respectively). The infants were both initially misdiagnosed as having eczema and infection. Premature infants are in negative zinc balance and though the additional factor of a low maternal breast milk zinc concentration may be necessary to provoke symptoms, rashes developing in such infants in the months following premature birth should raise the suspicion of zinc deficiency.

Zinc deficiency in infancy may occur in several circumstances. Best known is acrodermatitis enteropathica, a rare autosomal recessive disorder of impaired zinc absorption that is characterized by failure to thrive, diarrhoea, sparse hair and a periorificial scaly erythematous rash.[1] Zinc deficiency with similar features has been reported in infants receiving long-term parenteral nutrition with zinc supplementation inadequate for their increased requirements.[2–4] However, zinc deficiency can also develop in preterm infants who are otherwise normal, producing a rash identical with that of acrodermatitis enteropathica.[5–11] Several factors may combine to cause this symptomatic zinc deficiency. Premature infants have low zinc stores[12] and a poor mucosal uptake of zinc, but in many cases the maternal breast milk zinc content has also been low.[10–11] We report two further cases of premature breast-fed infants who developed symptomatic zinc deficiency 2–3 months after birth.

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CASE REPORTS

Case 1

A male infant (1640 g) was born at 31 weeks following spontaneous rupture of membranes to a healthy primigravida. There were no significant perinatal problems. He was established on breast feeding, and discharged at 5 weeks. At 9 weeks, he developed a rash in the napkin area, followed by a red scaly rash round the mouth which spread quickly over the face and neck. Neither rash responded to successive treatments with topical hydrocortisone, nystatin, and triamcinolone, and oral flucloxacillin. In view of maternal anxiety, he was admitted at 13 weeks, at which time the lesions affected the cheeks, behind the ears, the neck, and the napkin area (Fig. 1). Weight and head circumference were above the third centile; the baby was feeding well, and examination was otherwise normal. *Staphylococcus aureus* was grown from skin swabs. He was treated with oral flucloxacillin and nystatin, topical potassium permanganate soaks and nystatin and hydrocortisone cream. He made good clinical progress, and was discharged after 6 days on emollients only, with the rash 'nearly clear'. Over the next 10 days the rash reappeared, accompanied by rhinitis and loose stools, and he was re-referred.

The serum zinc assay result, which had been requested during admission, became available at this stage and was found to be low at 4·8 μmol/l (reference range 11·5–24·5 μmol/l). A second specimen was 3·0 μmol/l. Oral supplementation with zinc sulphate 50 mg/day was instituted.

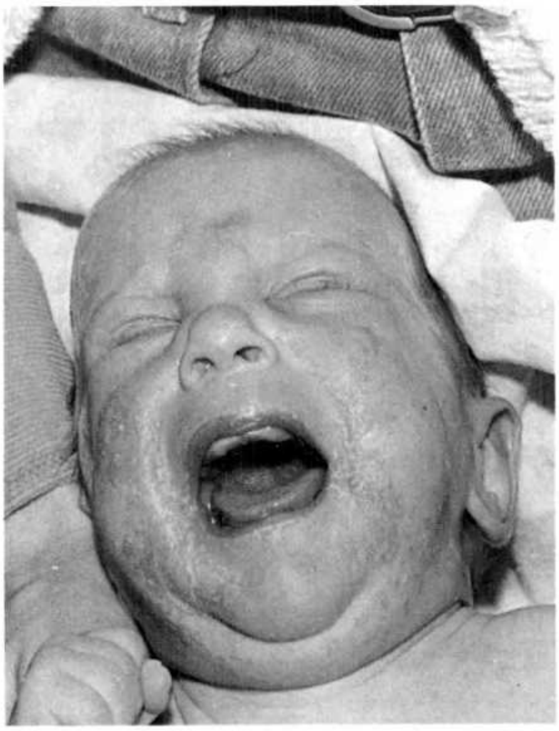

FIGURE 1. Case 1. Scaly erythema of face prior to zinc supplementation. Note that the facial rash extends only to the angles of the mouth.

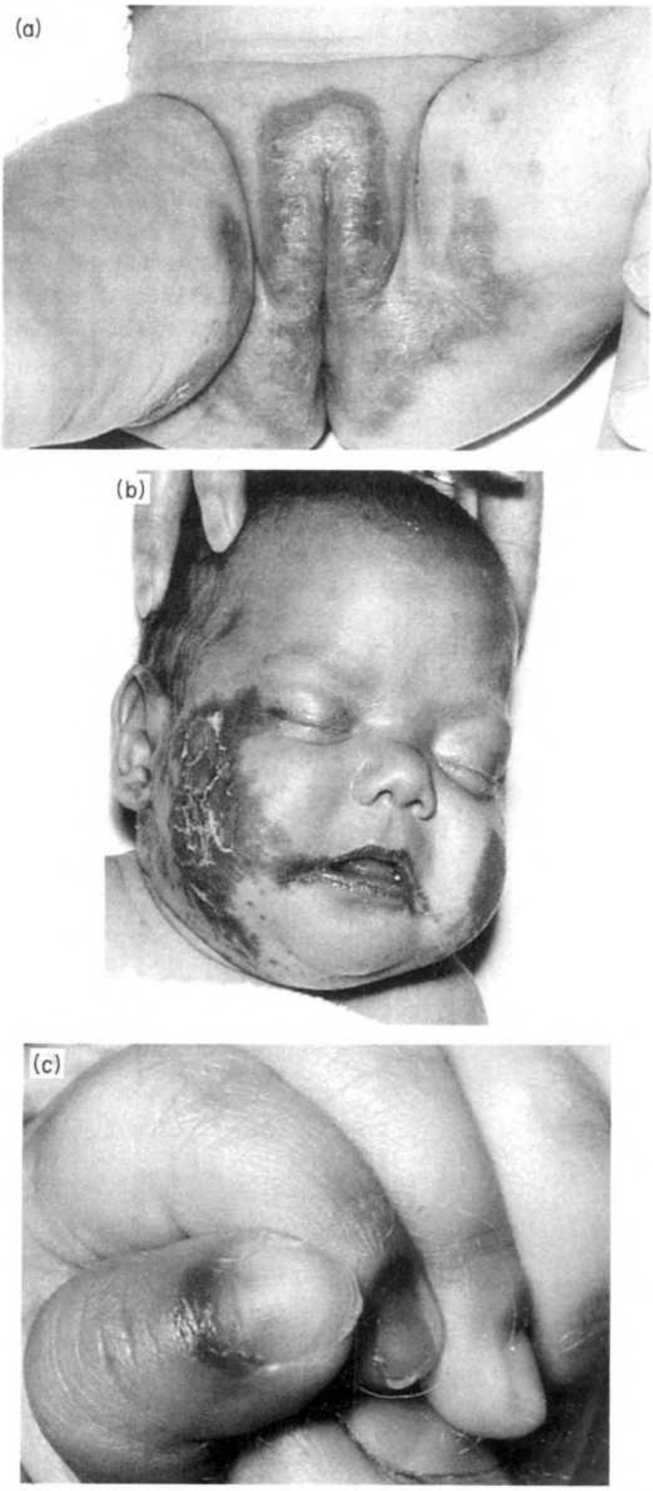

FIGURE 2. Case 2. Involvement of napkin area with sparing of flexures (a), rash on cheeks extending to angles of eyes and mouth (b) and also paronychia (c) at presentation.

The rash cleared within 48 h, and his parents reported that he became more alert, with improved feeding and firmer stools. He had been breast-fed throughout; maternal serum zinc was 10·5 μmol/l and breast-milk zinc, measured 21 weeks after delivery, was 2·3 μmol/l. The infant's serum zinc was maintained in the normal range by supplementation, but remained normal when this was discontinued at 21 weeks. He remained well and at age 18 months growth was satisfactory on the third centile for weight and on the tenth for length.

Case 2

A female infant (1360 g) was born at 28 weeks of gestation to a healthy woman with one previous normal full-term infant. The first week was complicated by streptococcal pneumonia and she recovered after ventilatory support, parenteral feeding and parenteral penicillin. Thereafter, she made steady progress on expressed maternal breast milk, with vitamin and iron supplements. Apart from a transient oral thrush, she was well after discharge until 13 weeks after birth when a papular eruption appeared on the right cheek. Despite initial treatment for eczema with 1% hydrocortisone, the lesions continued to extend. Oral erythromycin was commenced for presumed staphylococcal sepsis, but when at 15 weeks the rash spread to the napkin area she was referred by her general practitioner for dermatological opinion.

At that time (Fig. 2a–c) there were confluent areas of erythema on the right cheek, with scaling and crusting which extended to the outer canthus and angle of the mouth. The upper and lower lips were spared. There was a less extensive papular rash on the left cheek. In the napkin region there were erythematous areas with superficial ulceration and satellite lesions. The flexures were spared. There was erythema of the nailfolds on the thumbs, index and middle fingers of both hands, and a solitary papule on the plantar surface of the left hallux. Head circumference was on the fiftieth centile, and weight above the tenth, for gestational age. The infant otherwise appeared clinically normal. Because of the suspicion of disseminated skin infection she was admitted and commenced on oral flucloxacillin and topical mupirocin. There was no improvement over 24 h, and bacteriological, mycological and virological specimens proved negative.

As the rash resembled that of case 1, a trial of oral zinc sulphate 50 mg/day was instituted. Serum zinc before treatment was 3·6 μmol/l (normal range 9–15 μmol/l). Maternal serum zinc was 13·2 μmol/l; breast-milk zinc was 3·2 μmol/l at 15 weeks following delivery. The clinical response over the following week was again dramatic and the rash cleared completely from the face and extremities, the napkin area healing more gradually. Zinc supplements have been discontinued without any relapse.

DISCUSSION

Although the rash can be identical, zinc deficiency in preterm infants is distinct from the autosomal recessive acrodermatitis enteropathica. In the latter, breast feeding is protective,[1] since the breast milk contains a factor that enhances zinc absorption,[13] and the disorder appears on weaning or in bottle-fed infants. In contrast, in transient symptomatic zinc deficiency the rash occurs in spite of breast-feeding.[5–11] Most cases have been associated with low breast-milk zinc content, and an identical rash in full-term infants has been associated with low breast-milk zinc.[14–15] Maternal breast-milk zinc levels were much lower in our cases than the 10–15 μmol/l which might be expected at this stage of lactation.[10,16] However, low breast-milk zinc content is not the sole cause of deficiency; even with normal maternal breast-milk zinc, premature infants are in negative zinc balance for the first 13 weeks of life, and mean serum zinc levels in these infants fall well below the normal range.[12]

In view of these findings, it is surprising that symptomatic zinc deficiency is not more common. Our cases suggest several reasons why mild forms of transient symptomatic zinc deficiency in preterm infants could go unrecognized. The rash may appear weeks after discharge, and other symptoms resembling acrodermatitis enteropathica are usually minor. Moreover, neither of our infants had a clearly periorificial rash; the facial lesions arose from and were centred on the cheeks, extending to only the angles of the mouth. In the napkin areas the immediate perianal regions were spared, as were the flexures. Both cases were misdiagnosed first as eczema, then as probable primary candidal or staphylococcal infection. Other cases have commonly been treated with antibiotics, antifungal agents, and hydrocortisone for several weeks before diagnosis.[11] In case 1 there was staphylococcal superinfection, and vigorous in-patient treatment with topical and oral anti-microbial agents, and hydrocortisone, resulted in an unambiguous improvement. Superinfection is common and an initial response to antibacterial agents, perhaps mediated by reduction in the local need for zinc, has been noticed before.[11] Despite inappropriate treatment, if the lesions resolve as zinc balance improves the diagnosis could be missed.

However, even transient zinc deficiency is important, since zinc is needed by many enzymes of growth and development,[17] and treatment is simple. While problems had not been noted beforehand, parents of both our cases reported an improvement in alertness and feeding following zinc supplementation. For premature infants, maternal breast-milk may be inadequate,[18] and its ability to prevent sub-clinical or symptomatic zinc deficiency cannot be assumed. Accordingly, those involved in the care of infants in the months after preterm birth should be aware that persistent napkin dermatitis, or red scaly rashes on the cheeks, neck and nailfolds, may indicate zinc deficiency.

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Farber's lipogranulomatosis in siblings: light and electron microscopic studies

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SUMMARY

Two cases of Farber's lipogranulomatosis in siblings are reported. The clinical features included contractures of the limbs with swelling of the joints and subcutaneous nodules and erythematous infiltrated plaques. On histology there were many large foam cells in the dermis, and electron microscopy showed numerous large cells with round cytoplasmic lamellar and microtubular bodies.

Farber's lipogranulomatosis is a rare, genetically determined metabolic disorder in which there is a deficiency of lysosomal acid ceramidase and an accumulation of ceramide. Two cases of Farber's lipogranulomatosis in siblings are reported and skin biopsies from their lesions were studied using light and electron microscopy.

CASE REPORTS

Case 1

A female born in 1983. The parents were not related. From the age of 3 months she developed contractures of the limbs and swelling of her joints with several subcutaneous nodules. By the age of 8 months, asymptomatic plaques had developed in the perianal region, on the back, on the occipital region and in the mouth. Later, firm subcutaneous nodules appeared around several joints. She had recurrent episodes of diarrhoea. Clinical examination revealed a poorly nourished 3-year-old girl, height 64 cm, weight 7200 g and head circumference 51·3 cm. She had a shrill voice but there was no mental retardation. The liver and spleen were not enlarged. There were painful contractures and swellings of the joints and subcutaneous nodules were noticed around joints of the fingers, wrists and ankles. The lower part of her face was swollen and

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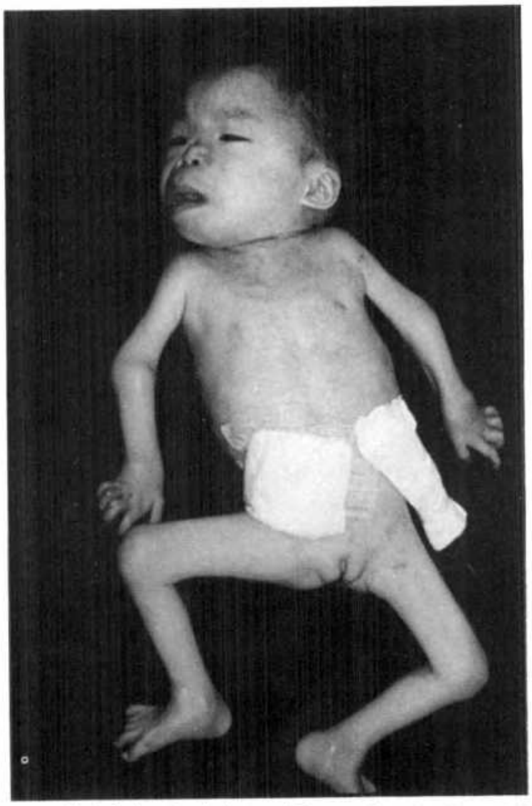

FIGURE 1. Case 1. A 3-year-old girl showing joint swellings and contractures, and subcutaneous nodules on the periarticular spaces.

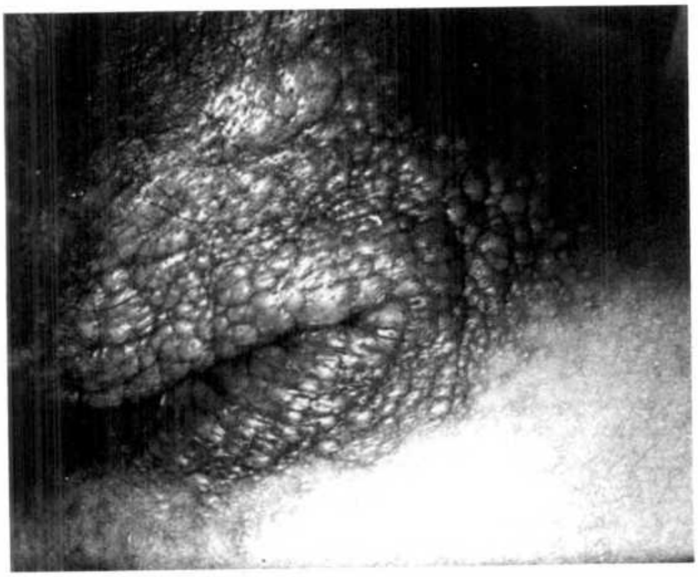

FIGURE 2. Erythematous infiltrated papules on the neck of case 1.

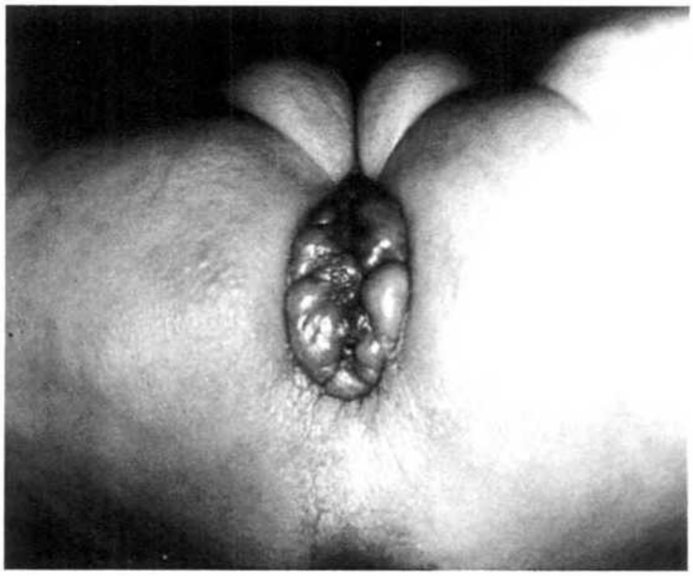

FIGURE 3. Perianal region of case 1. Soft erythematous masses in the perianal area.

deformed due to hypertrophic gingiva (Fig. 1) and there were erythematous nodules that involved the oral mucosa. Erythematous papules that developed into confluent plaques were present on the neck and back (Fig. 2). Similar lesions were present in the perianal region (Fig. 3). The level of IgE was raised, 2400 IU/ml and X-rays of the joints showed contractures and soft periarticular swellings.

Case 2

The younger brother of case 1 was born in 1985. At birth, rather stiff shoulder and ankle joints were observed and at the age of 6 months there were erythematous skin lesions in the perianal region. He then followed a similar clinical course to his sister. The contractures of the limbs and joint swellings with subcutaneous nodules and erythematous plaques of the skin were similar to those seen in his sister.

METHODS

Skin biopsies were taken from lesions on the neck, back and perianal area in case 1. Sections of paraffin-embedded tissue were cut and stained with haematoxylin–eosin, periodic acid Schiff (PAS), colloidal iron stain (Müller–Mowry's method) and in addition frozen sections were stained with Sudan III, Sudan black and oil red O. For electron microscopy, small blocks of tissue were fixed and stained in 2·5% cacodylate (0·1 M)-buffered glutaraldehyde (containing 0·1% ruthenium red-Chroma) at pH 7·4 and kept at 4°C overnight.[1] They were then rinsed for several hours with the same buffer and post-fixed and stained in 2% buffered OsO_4 (containing 0·1% ruthenium red) at 4°C overnight, rinsed for several hours, dehydrated, and embedded in Epon 812. Ultra-thin sections were then cut and viewed in the electron microscope.

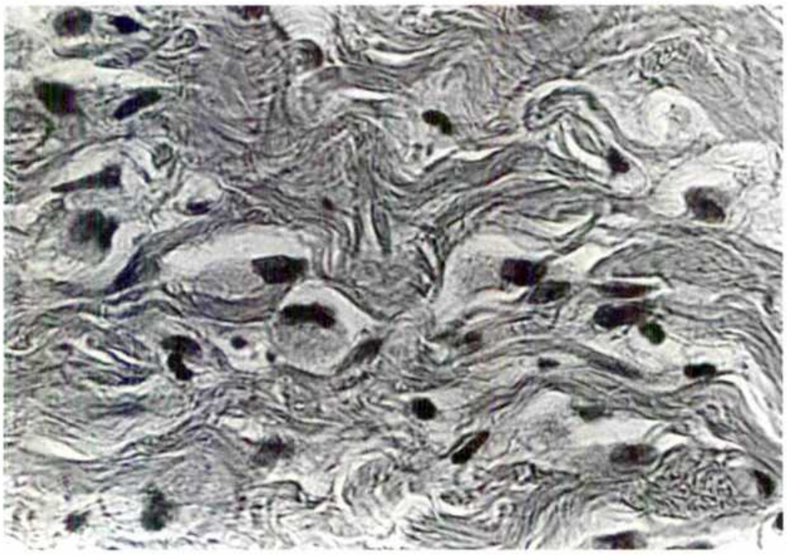

FIGURE 4. Large cells with foamy cytoplasm in the dermis (H&E × 580).

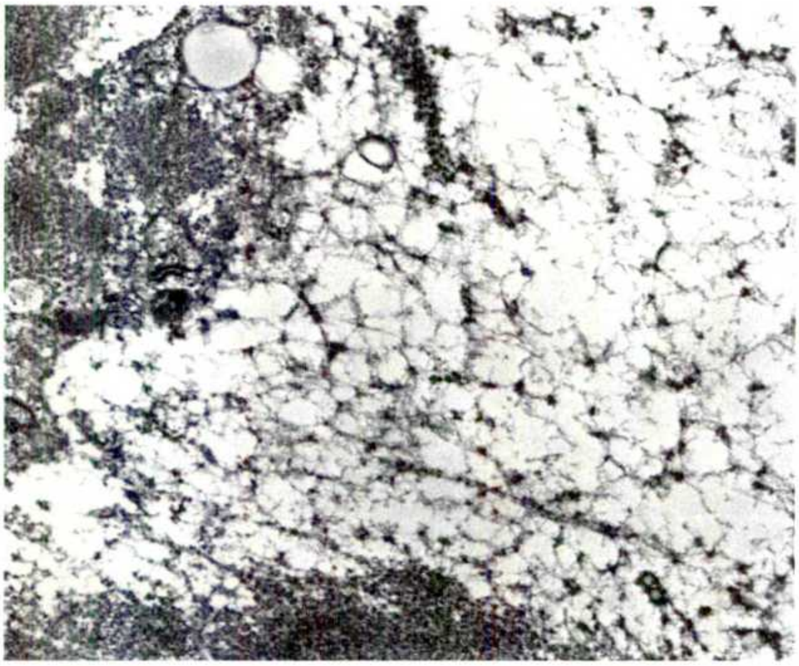

FIGURE 5. An electron micrograph stained with ruthenium red shows abundant network of mucopolysaccharides throughout the dermis. (× 1200).

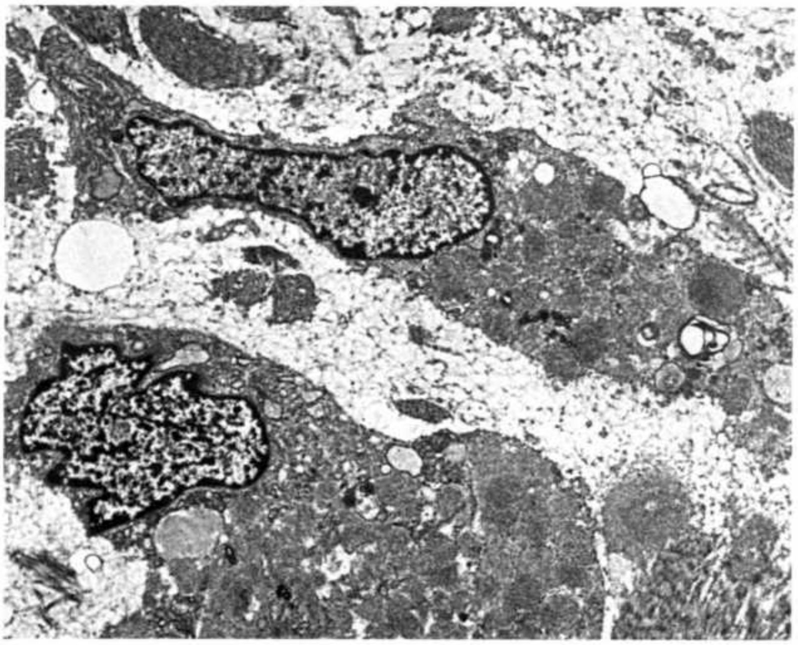

FIGURE 6. Many large cells in the dermis with intracytoplasmic bodies (× 4500).

RESULTS

Light microscopy

The findings were much the same in the biopsies taken from all three areas except that in the perianal region there was prominent telangiectasia with a proliferation of capillaries. Large cells with a foamy cytoplasm were seen around the capillaries and among the collagen in the dermis (Fig. 4). The cells had a large nucleus and the foamy cytoplasm was PAS positive and stained with colloidal iron using the Müller–Mowry method, but did not stain with Sudan III, Sudan black and oil red O. Colloidal iron-positive staining was also present between the separated collagen bundles.

Electron microscopy

Using ruthenium red this revealed an abundant network of mucopolysaccharides throughout the dermis (Fig. 5). Numerous large cells were present and these cells were considered to be the foam cells as seen on light microscopy and were probably fibroblasts (Fig. 6). Some of the cells contained only a small amount of intracytoplasmic material that consisted of round lamellar and microtubular bodies. Many cells were, however, full of this material and these lamellar and microtubular bodies became confluent (Fig. 7).

DISCUSSION

Farber's lipogranulomatosis[2] is recognized as an autosomal recessive disorder characterized by a deficiency of lysosomal acid ceramidase[3] and with the accumulation of tissue ceramide. The characteristic clinical features[4] are painful and progressively deformed joints, subcutaneous nodules, progressive hoarseness, nutritional failure and in some cases central nervous system

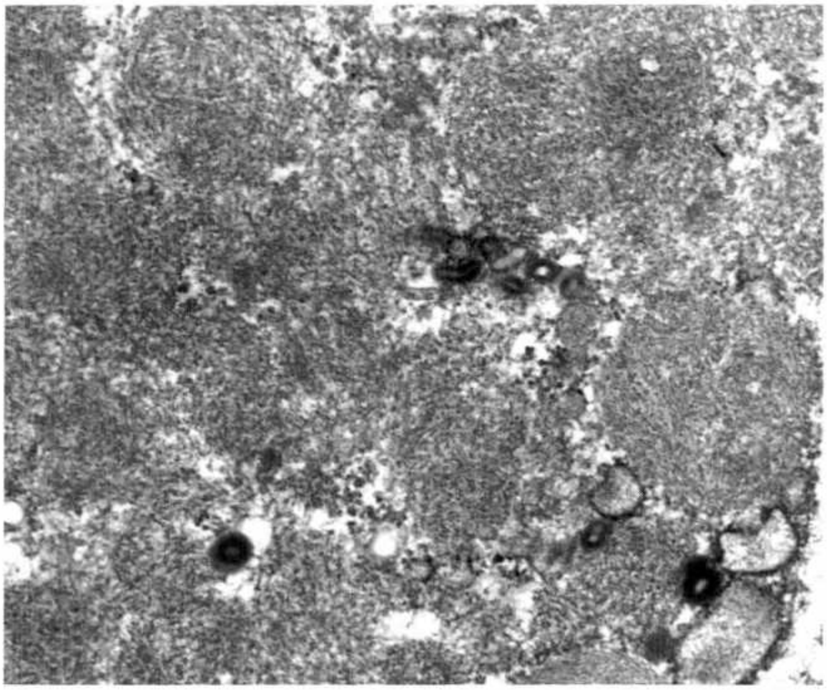

FIGURE 7. Many cells are full of round shaped bodies containing lamellar or microtubular structures. (× 16 500).

involvement. The lungs, heart, and lymph nodes may also be affected. On light microscopy foam cells are found in the early lesions and granulomas in the more advanced nodules. The diagnosis is determined by finding defective acid ceramidase activity in cultures of skin fibroblasts or of leucocytes.

Our cases both had the characteristic clinical and histopathological findings of Farber's lipogranulomatosis. However, we were not able to detect whether there was a deficiency in acid ceramidase. In addition our cases had perianal telangiectasia plaques that appear to have been not previously reported. Many foam cells with an associated telangiectasia were seen in the biopsy taken from this perianal region.

There have been several previous electron microscopical studies and abnormal inclusions have been found in the cytoplasm of myelinated nerve fibres and in histiocytes,[5] and Zebra bodies in endothelial cells and numerous curvilinear bodies in fibroblasts.[6] Curvilinear bodies (Farber's bodies) have been described in the cytoplasm of fibroblasts.[7] In another study numerous membrane-bound inclusions were found in epidermal cells.[8] Our ultrastructural studies revealed an abundant accumulation of mucopolysaccharides in the dermis and the cytological features of the fibroblasts were similar to those previously described. However, involvement of the nerves and the presence of curvilinear profiles were not observed. This may be a result of a difference in the stage of the disease.

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Thallium poisoning: a dermatological perspective

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SUMMARY

The dermatological findings in five patients with thallium poisoning are described. Cutaneous involvement consisted mainly of an acneiform eruption, pellagra-like dyssebacea, crusted eczematous lesions and dry scaling of the distal parts of the extremities. Alopecia was not evident initially but later became apparent. The patients also had gastrointestinal and neurological symptoms and signs.

Thallium administration in man has a notorious history, and although initially the drug of choice in the treatment of certain skin diseases, particularly fungal infections, it soon became obvious that thallium could cause serious side-effects and death. Because it is colourless, odourless and tasteless, it superseded arsenic as a homicidal poison during the 1950s,[1] and in most countries is now strictly regulated. Recent reports of thallium poisoning are rare and the cutaneous manifestations are seldom described. However, a modern-day development is the use of thallium as an adulterant in the drug trade.[2]

CASE REPORTS

Five previously healthy young men were referred to us from an outlying hospital in a neighbouring country to South Africa. One of the patients was stuperose, the others complained of diffuse muscle and joint pains, loss of appetite and abdominal cramps. One complained of hyperaesthesia of the palms and soles and another of neuralgic pain in the legs. Due to language problems more detailed histories were not obtained.

On admission all the patients had mild to severe stupor with a motor and sensory peripheral neuropathy and weakness of the legs. The neurological and cutaneous lesions became worse after admission and loss of pain and light touch sensation in a stocking distribution developed in all the patients. In one patient cranial nerve palsies developed, particularly involving C3 and C4 and two of them were transferred to the intensive care unit because of respiratory failure and severe coma.

There was follicular plugging of the skin by inspissated keratinous material, particularly on the nose and cheeks and in the nasolabial folds (Table 1). Crusted eczematous lesions were present in four of the patients, three of whom also had an acneiform eruption (Fig. 1). Fine dry

TABLE 1. Clinical symptoms

	Patient				
	A	F	N	P	H
Alopecia					
Scalp	±	±	+ +	+ +	+ +
Eyebrows	±	±	+	+	+
Eyelashes	−	−	−	+	−
Limbs	−	−	+	+	+
Follicular plugging	+ Cheeks	+ Lip, nose, nasolabial folds	+ + Chin, nasolabial, eyebrows	+ Chin	+ Nose, chin
Pustular dermatosis	+ Cheeks	−	+ + Chin, cheeks	−	+ + Cheeks, chin, arms, and legs
Dermatitis	+ Scalp, ears	+ + Scalp	+ + Scalp, eyebrows, cheeks, chin	−	+ Scalp
Scaling of palms and soles	−	+ +	+ +	+	+ +

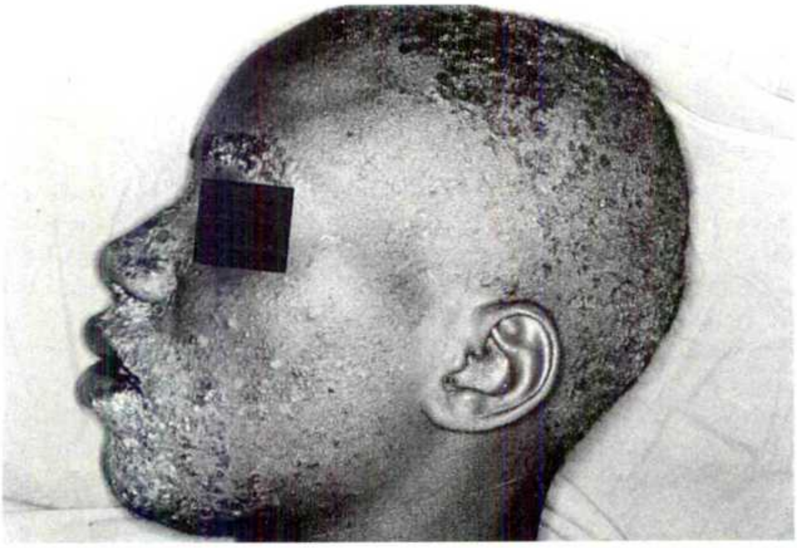

FIGURE 1. Heavily crusted dermatitis of eyebrows, nose and around mouth, lesser involvement of scalp. Acneiform pustular eruption over cheeks and chin. Loss of hair evident.

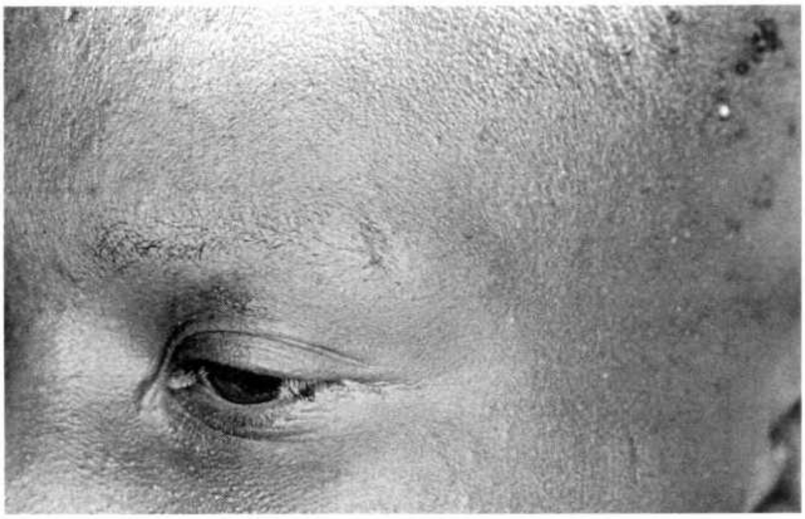

FIGURE 2. Alopecia of lateral aspect of eyebrows; medial aspect relatively spared. Loss of eyelashes. Note only few remaining tufts in peripheral margin of scalp hair.

scaling was present over the palms and soles in four patients. Alopecia was not evident on admission, but developed soon afterwards. The hair could be pulled out easily and later on it fell out spontaneously. In one patient there was such extensive alopecia that only a thin peripheral rim of hair remained on the scalp. In three patients the hair loss involved not only the scalp, eyelashes and lateral aspects of the eyebrows (Fig. 2), but also the hair on the arms and legs.

The diagnosis was uncertain until the first patient developed alopecia and then the possibility of thallium poisoning was considered. In all five patients the urinary and plasma levels of thallium exceeded 500 mg per dl. The patients all came from the same remote village and it is presumed they were poisoned with contaminated food.

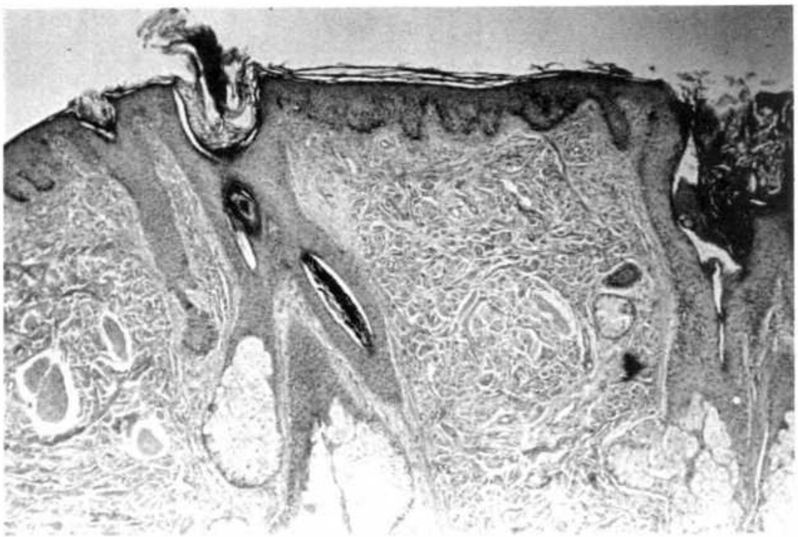

FIGURE 3. Hyperkeratosis and focal parakeratosis, follicular plugging and disintegrating hair shafts. (H & E × 43·5).

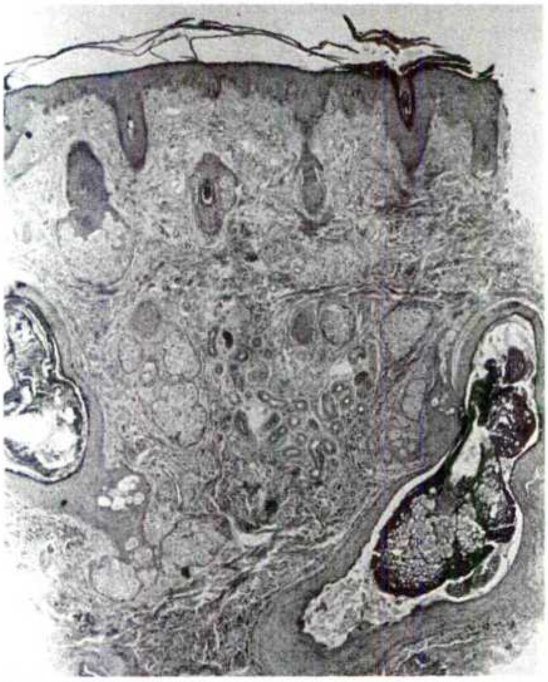

FIGURE 4. Cyst-like dilatation of hair follicles, the bigger one containing an extruded necrotic sebaceous gland. (H & E × 67)

Investigations revealed no significant change in the blood count although 4 weeks later one patient developed a normocytic, normochromic anaemia. All the patients had disturbed liver function tests that returned to normal before their discharge from hospital.

Skin biopsies were taken from the scalp, from acneiform pustules on the face and the hyperkeratotic lesions of the feet. The histology of the lesions on the scalp and cheek showed similar features, of gross follicular plugging, with eosinophilic keratohyaline granules in the adjacent granular layer of the epidermis. Most of the hairshafts were disintegrating (Fig. 3). Many follicles showed cyst-like dilatation and some contained extruded and necrotic sebaceous glands (Fig. 4). Biopsies of the pustular lesions on the face showed a folliculitis and perifolliculitis with necrosis of the follicles. Skin biopsies from the feet revealed marked hyperkeratosis and hypergranulosis with evidence of disturbed keratinization. There was a proliferation of thick-walled blood vessels in the upper dermis.

All the patients were treated with a forced diuresis with Prussian Blue and Mannitol, as well as being nursed in the intensive care unit. After the acute phase they were given physiotheraphy. Four of the five made an uneventful recovery but the fifth has permanent neurological damage.

DISCUSSION

Thallium was first used to reduce night sweats in tuberculosis, but when it was found to cause alopecia it was used as an epilating agent for fungal infections of the scalp. Serious side-effects were soon noted[3] but it continued to be used in the treatment of tinea capitis and fungal infections.[4-6] Dermatological use was later confined to a topical cream, but even this produced serious side-effects.[7] Thallium has been used as a rodenticide as recently as 1980, but this is now prohibited. However, it is still widely used in industry as thallium oxide, particularly in the manufacture of optical glass.

The mean lethal dose of thallium in an adult is about 1 g of thallium sulphate, and single doses of 5 mg/kg body weight have caused toxic reactions in children.[8] Subacute and chronic intoxication is associated with accumulative tissue retention, and any sudden release from the tissues may produce acute toxic symptoms. Thallium is apparently transported into cells by the potassium pump mechanism[9] but is less readily released than potassium. It is excreted largely in the urine and partly in bile.

The clinical findings in thallotoxicosis have been the subject of several reviews.[7,8,10–12] The onset of symptoms is usually insidious, reaching a maximum in the second or third week followed by gradual subsidence or death. Vomiting or merely nausea may be experienced followed by constipation. After 3–4 days, or sometimes after a prolonged period of latency, severe neuralgia develops and later retrosternal and abdominal pain, together with excessive thirst and restlessness. The patient may seem hysterical, and there may be a painful glossitis with redness of the tip of the tongue and dark pigmentation around the root of the hair. Gradual development of tachycardia and progressive polyneuritis is followed by progressive dementia and epileptic seizures may occur.

In the first week there is often sudden onset of acne, and the hair loss develops in the second week involving the scalp, axillary and pubic regions and the lateral parts of the eyebrows. The sweat glands and the sebaceous glands are destroyed and the skin becomes dry and slightly scaly. The acne may be severe and necrotic lesions occur. The growth of the nail is impaired with the development of Mees' lines.

Hair loss is invariable in thallium poisoning,[12] and it has been suggested that this could be an indirect effect of thallium on the endocrine glands and nervous system.[13] However our study, as in a previous report on dogs,[14] indicates that thallium has a toxic effect on the hair follicle.

The patients reported had typical features of thallium poisoning and this diagnosis should be considered in patients who develop a sudden diffuse type of alopecia with neurological symptoms and signs.

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Proliferating tricholemmal tumour with lymph node metastases

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SUMMARY

A 42-year-old woman with a proliferating tricholemmal tumour (PTT) with regional lymph node metastases is reported. Histochemical studies showed evidence of tricholemmal keratinization. There was recurrence following excision and subsequently the patient developed lymph node metastases.

Proliferating tricholemmal tumour (PTT) is an uncommon neoplasm that usually occurs on the scalp of elderly women. These cases have been reported with different names: proliferating epidermoid cyst,[1] invasive pilomatrixoma,[2] tricho-chlamydocarcinoma,[3] giant hair matrix tumour,[4] proliferating tricholemmal cyst,[5] keratinizing tricholemmoma[6] and proliferating tricholemmal tumour.[7] To our knowledge, only three of these cases have developed metastases.[3,8,9]

We wish to report a case of PTT occurring in a middle-aged female with a long history of clinical recurrences and with regional lymph node involvement.

CASE REPORT

A 42-year-old lady was first seen in March 1982 with a red, firm nodule with irregular borders that measured 2 cm in diameter on the left side of the occipital region of her scalp (Fig. 1). This had progressively enlarged during a pregnancy one year previously. The lesion was excised, and on histology showed well circumscribed lobular proliferations of squamous epithelium surrounded by connective tissue septa (Fig. 2). Palisading of the nuclei of the basal cells of the tumour lobules was infrequently seen. The stroma was fairly cellular with an occasional glassy appearance around the lobules. A few dyskeratotic cells were seen in the epithelial lobules, and occasional mitotic figures (Fig. 3a). There were areas with clear cells that were PAS-positive

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(Fig. 3b). In some of the centres of the epidermoid lobules there were eosinophilic amorphous masses with evidence of calcification. There was no evidence of a pre-existing cyst.

A fluorescent thiol reagent, *N*-(7-dimethyl-amino-4-methyl-coumarinyl) maleimide (DACM), that binds sulphydryl groups,[10] showed strongly reactive areas for disulphide bond linkages in the fields that corresponded to the eosinophilic masses (Fig. 4a). Sulphydryl groups (-SH) were found around the epithelial cells either on the cell membrane or in the intercellular

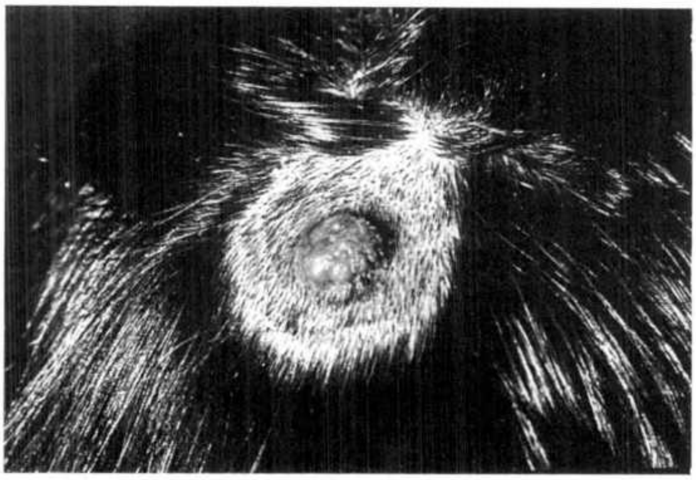

FIGURE 1. Nodule in the occipital region of the scalp.

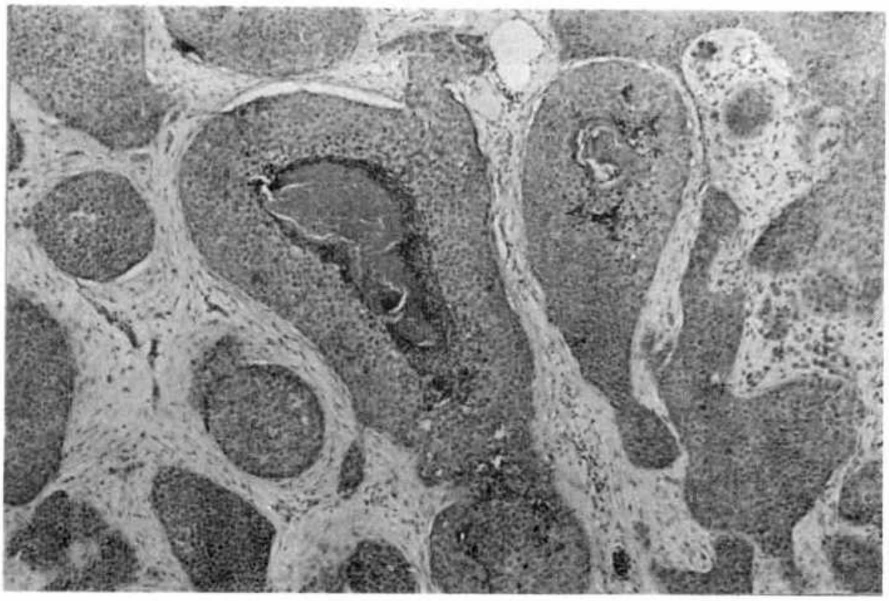

FIGURE 2. Lobular proliferations of squamous epithelium showing tricholemmal keratinization (Haematoxylin and eosin, × 85).

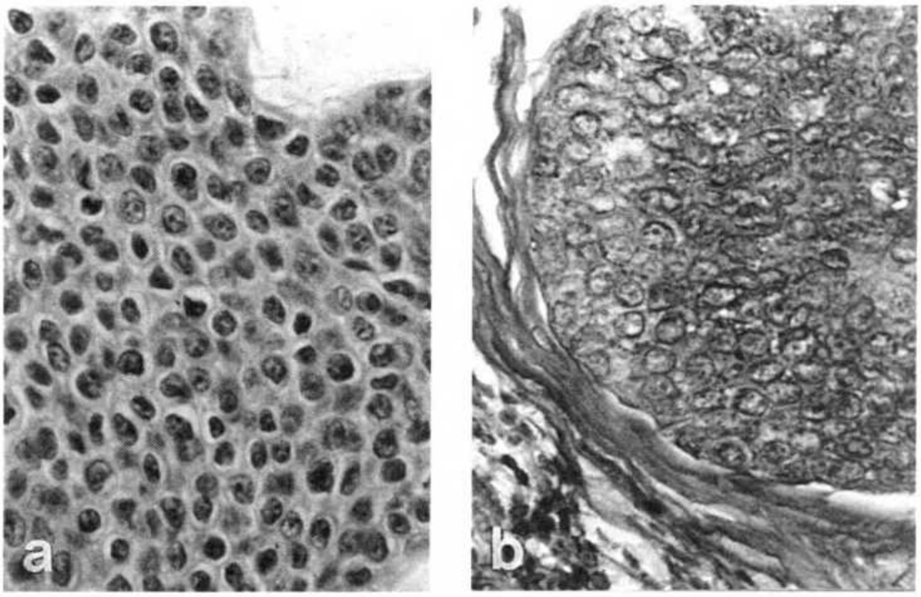

FIGURE 3. (a) Higher magnification showing slight atypia, dyskeratosis and mitotic figures (Haematoxylin and eosin, × 213). (b) PAS-positive cells in the epithelial lobules (Periodic-Acid-Schiff, × 213).

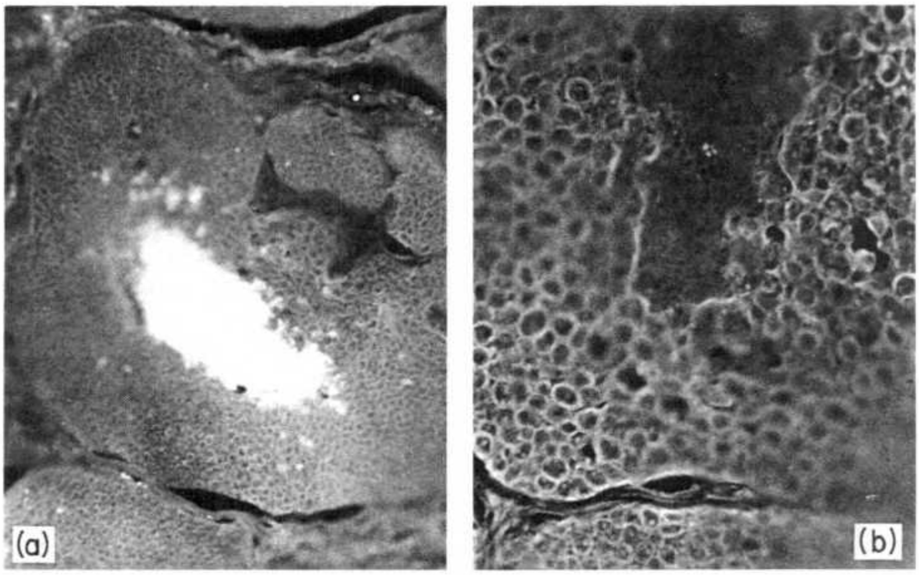

FIGURE 4. DACM staining showing (a) presence of S–S linkages (× 85) and (b) absence of -SH groups in the keratinized area (× 213).

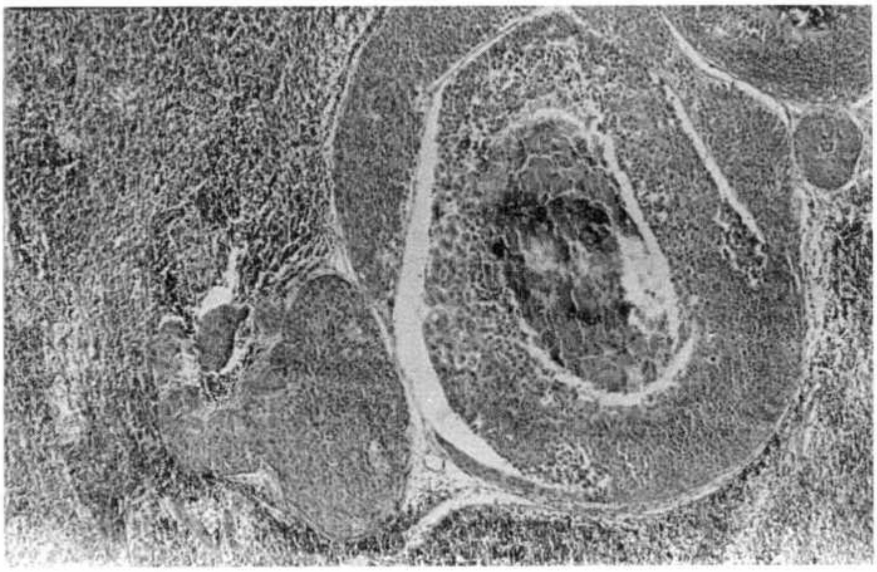

FIGURE 5. Lymph node metastasis showing evidence of tricholemmal keratinization (Haematoxylin and eosin, × 85).

spaces using this method (Fig. 4b). The strong fluorescence for these -SH groups was similar to the intensity seen in the epidermis and outer hair-root sheath.[11] A diagnosis of proliferating tricholemmal tumour was made. A CT scan of the skull and a chest X-ray were normal. The electrocardiogram showed slight left ventricular strain.

There was some recurrence in the same area of the scalp 6 months later when she was pregnant again, and this nodule was excised. The histology was that of PTT. The pregnancy resulted in a spontaneous abortion. In December 1983 there was a further recurrence, and by May 1985 this tumour was 3 cm in diameter. It was excised, and 4 months later an enlarged cervical lymph node on the left side of the neck was removed and found to contain tumour metastases. Further lymph nodes were removed from the left side of her neck, and three of these were found to contain epithelial metastases that showed evidence of tricholemmal keratinization (Fig. 5).

In December 1986 a further large tumour developed rapidly in the same area of her scalp, and on histology was that of PTT.

DISCUSSION

Proliferating tricholemmal tumour is a rare neoplasm more common in elderly women than men and is usually localized to the scalp. The tumour shows tricholemmal keratinization with abrupt formation of compact keratin without the formation of a granular layer. The tumour is often slow-growing, and though local recurrences are common, metastases are rare. The histological features of our case were typical, but the unusual aspects were the relatively young age of our patient, the aggressive growth of the tumour and the subsequent metastases to the regional lymph nodes. The prognosis of patients with PTT is good, even when there is, as in our case, lymph node involvement,[3,8,9] and distant spread does not occur.

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Society Proceedings

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HOST: PROFESSOR B.J.VERMEER

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CASE REPORTS

Ki-1-positive T-cell lymphoma in a female patient with 'Hodgkin's disease' in the past

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History and examination. A 19-year-old female patient was diagnosed in 1984 as having a nodular, sclerosing Hodgkin's disease stage IIIA. Treatment consisted of polychemotherapy with MOPP and radiotherapy and a complete remission was achieved. In April 1988, she presented with a tumour on the lateral side of the right upper leg. The tumour was 3 cm in diameter with central ulceration. In the right inguinal area there was a small palpable lymph node.

Histopathology and immunohistochemistry. On histology the tumour showed an atypical lymphoreticular infiltrate that consisted of large cells with sometimes small nucleoli. There were many mitoses and eosinophils were present. The atypical cells were $CD2^+$, $CD4^+$, $CD5^+$, $CD8^-$, $CD30(Ki\text{-}1)^+$, Leu-$M1^-$. Re-examination of a lymph node excised in 1984 showed similar histological and immunohistochemical findings as the recent skin biopsy. The initial diagnosis of Hodgkin's disease was rejected and the diagnosis was changed into Ki-1^+ T-cell lymphoma with lymph node involvement, the recent skin tumour being a relapse of the lymphoma that was diagnosed in 1984.

Discussion. In 1985 Stein *et al.*[1] described a large-cell lymphoma of activated lymphoid cells (mainly of T-cell origin) expressing a Hodgkin's disease-associated antigen, Ki-1. This Ki-1^+ large-cell lymphoma (or large cell anaplastic lymphoma) may be difficult to distinguish from Hodgkin's disease. Additional immunohistochemical studies are of help in differentiating Ki-1-positive large-cell lymphoma from Hodgkin's disease. Ki-1^+ large-cell lymphoma of the skin without lymph node involvement generally has a very favourable prognosis, whereas when there is primary lymph node involvement the prognosis is poor. Of 10 patients with a primary cutaneous $\mathrm{K1}^+$ T-cell lymphoma registered at the Dutch Lymphoma Working Group nine are in complete remission with a mean follow-up of 32 months.

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Lichen aureus with underlying arteriovenous shunt

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History. A 10-year-old girl presented with a progressing purple patch on the right medial malleolus which she had had for 4 years. She did not complain of pain or itch.

Examination. A sharply demarcated purple patch 6 cm in diameter was present on the right medial malleolus (Fig. 1).

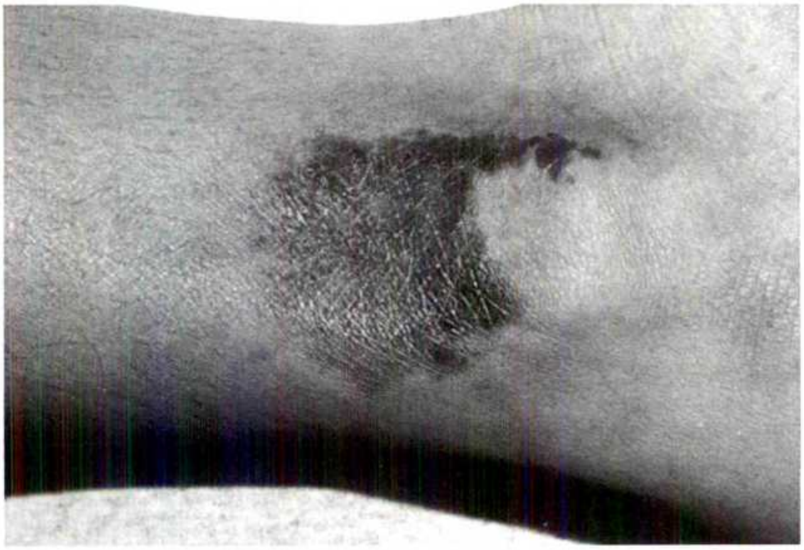

FIGURE 1. Purple patch on right medial malleolus.

Histology. There was a thin and slightly hyperkeratotic epidermis with a band-like inflammatory cell infiltrate in the upper dermis, with prominent capillaries and with extravasation of erythrocytes. There were deposits of iron extending into the deep part of the dermis.

Light reflex rheography (LRR) of the right leg showed a reduced refill time that could be completely corrected by applying compression over the affected area.

Doppler ultrasound of the purple patch showed a venous vessel sound, accompanied by a soft heartbeat sound, as can be heard over small calibre arteriovenous shunts.

Discussion. Although suggested by Shelley *et al.*,[1] this is the first time to our knowledge that a vessel anomaly could be determined by LRR and Doppler investigation and in close relationship to a patch of lichen aureus.

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Primary cutaneous actinomycosis

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History. In 1986 a 29-year-old man was admitted with a 2-year history of recurrent abscesses and draining sinuses of his right leg. Treatment with antibiotics had, in the past, given only an incomplete and temporary improvement. The bacterial cultures were negative.

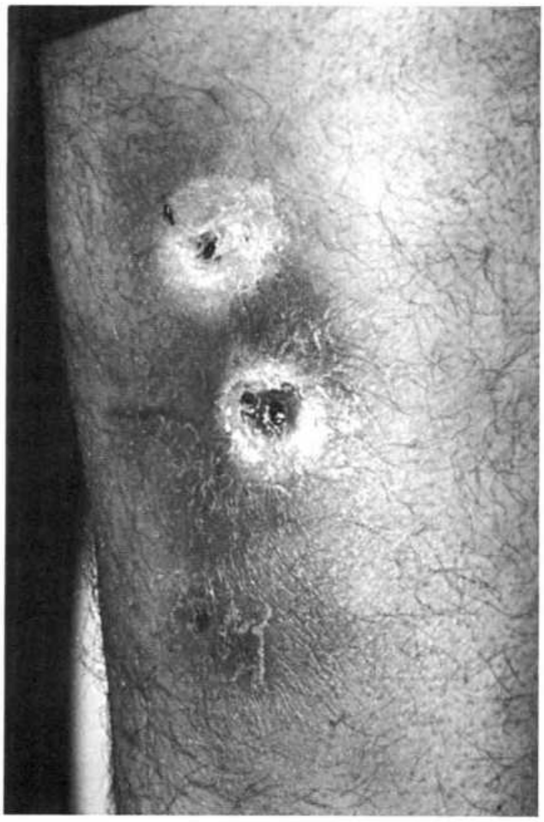

FIGURE 1. Infiltrated area with draining sinuses on right upper leg.

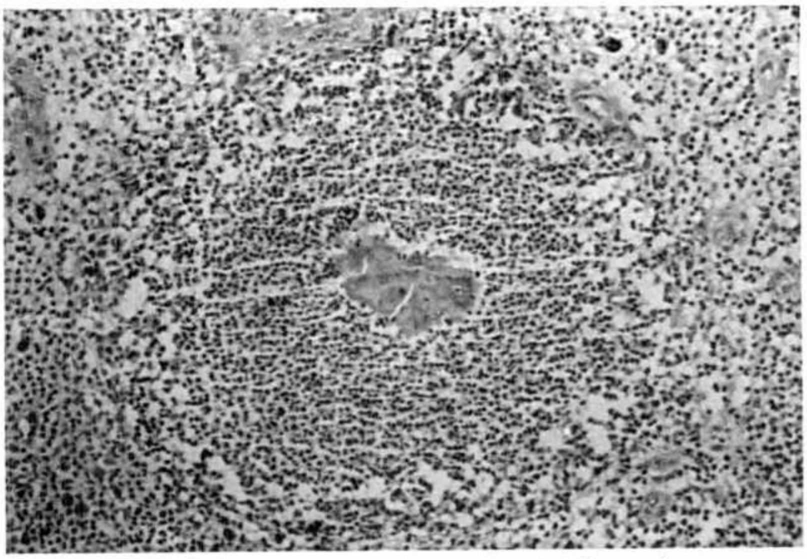

FIGURE 2. Polymorphonuclear cell infiltrate with typical filamental structures.

Examination. There was a red–brown infiltrated area of 15 cm on the right upper leg with several draining sinuses (Fig. 1). On the skin of the medial malleolus and above the lateral malleolus identical but smaller lesions were seen. Further physical examination revealed only many carious teeth. The chest X-ray was normal.

Histology. This showed a polymorphonuclear cell infiltrate with many filamental structures typical for actinomycosis (Fig. 2). Bacterial cultures yielded *Actinobacillus actinomycetemcomitans*.

Diagnosis. Although actinomyces was never cultured from the exudate the diagnosis cutaneous actinomycosis was made on (a) the filamentous organisms seen[1]; (b) the culture of *Actinobacillus actinomycetemcomitans*, an organism frequently accompanying actinomycoses,[2,3] and (c) the clinical picture of recurrent abscesses with draining sinuses.[4,5]

Treatment. The patient was treated with high doses of intravenous penicillin for 6 weeks and then given oral penicillin for a further 3 months. Many teeth were extracted. Once penicillin was commenced the skin improved and after 1 year the patient was asymptomatic.

Discussion. Actinomycosis is a chronic granulomatous disease caused by the Gram-positive filamentous anaerobe *Actinomyces israelii*. The bacteria were originally misclassified as fungi.[1] Very often Gram-negative bacteria can also be cultured and in most cases *Actinobacillus actinomycetemcomitans*, and this may play a synergistic role.[2,3]

Our patient suffered from the primary cutaneous form which is an uncommon type.[4,5]

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Association of generalized granuloma annulare with autoimmune thyroiditis

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History. In 1987 a 59-year-old woman was admitted with a 12-year history of skin lesions that were previously diagnosed as granuloma annulare. The lesions had recently spread over the trunk and arms. She had a past history of primary autoimmune hypothyroidism and had been treated with thyroid preparations.

Examination. There were many red-brown and skin-coloured papules and annular lesions on the trunk and to a lesser extent on the legs. The diameter of the lesions varied from 0·2 to 1 cm.

Histology. In the dermis were multiple granulomatous foci with multinucleate giant cells. The centre of these foci showed degeneration of the collagen.

Treatment and clinical course. Because the patient had noticed improvement of her skin lesions with sun-exposure she was initially treated with UVB. The skin lesions improved but later spread and this coincided with a reduction of the dose of her thyroid medication.

Discussion. The association of generalized granuloma annulare with autoimmune thyroiditis has been described.[1,2] Over a period from 1983–87 50 patients (43 female and seven male) with granuloma annulare have attended our out-patient clinic. Of these, 42 had localized granuloma annulare. The associated diseases in these patients were: two with insulin-dependent diabetes mellitus[3] and two patients with a non-specific arthritis. Eight patients had generalized granuloma annulare, in three there was an associated autoimmune hypothyroidism,[1] one had rheumatoid arthritis, one patient also had infectious mononucleosis and one had diabetes mellitus. This retrospective study supports an association of generalized granuloma annulare with autoimmune thyroiditis.

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Familial leiomyoma cutis

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History. A 55-year-old woman had since the age of 28 gradually developed multiple tumours on the legs and arms, which were occasionally painful on mild pressure or in cold weather. She had had three of these lesions removed and had undergone a hysterectomy.

Examination. On the right shoulder, upper arm and on both thighs there were numerous skin coloured and red-pink papules and nodules that were mostly distributed in groups (Fig. 1).

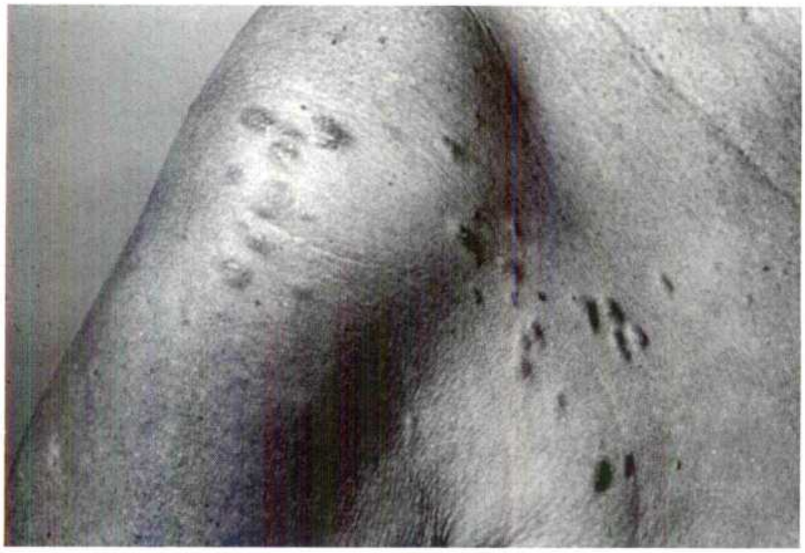

FIGURE 1. Papules and nodules on the right shoulder.

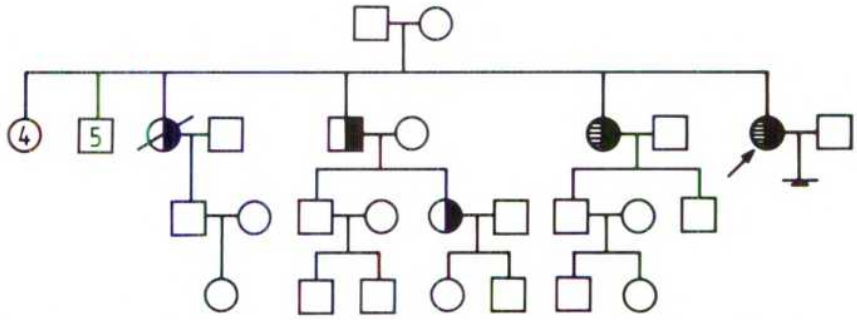

FIGURE 2. Family history of patient. Cutaneous leiomyomas are presented black on the right half of the symbols and horizontal black stripes on the left half represent uterine leiomyomas.

Histology. There were interlacing mature smooth muscle bundles in the dermis and the histology was consistent with the diagnosis of leiomyoma cutis.

Family history. Similar skin lesions were observed in other members of the family and a sister had had a hysterectomy (Fig. 2).

Discussion. The occurrence of leiomyoma cutis in more than one member of a family has been rarely documented.[1] These benign tumours may be solitary or in clusters and can be classified into non-hereditary and hereditary familial types. Female patients with cutaneous leiomyomas, especially those with a positive family history, should have a gynaecological examination.[2] In contrast to the skin lesions, leiomyosarcomas can arise in the uterine leiomyomas.

The treatment of symptomatic cutaneous leiomyomas is excision of the more painful lesions and analgesics to control the pain. Various other pharmacological treatments have been reported to be effective in some cases.[3] The ice cube test is a simple method to assess the potential benefits of these treatments for pain and may be a useful aid in diagnosis.[3]

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Kaposi's sarcoma in a renal transplant recipient

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History. A male, aged 43 years was born in Morocco and underwent a renal transplant in 1984. The immunosuppressive regimen consisted of azathioprine 100 mg/day in combination with prednisone 12·5 mg/day and early in 1985 purplish, nodular lesions appeared on the trunk and extremities.

Examination. There were purplish, nodular and flat lesions on the left upper arm, on the abdomen in a surgical scar and on the left lower leg (Fig. 1). Visceral involvement with Kaposi's sarcoma was excluded.

Laboratory tests. No serological evidence for recent cytomegalovirus or HIV infection was found.

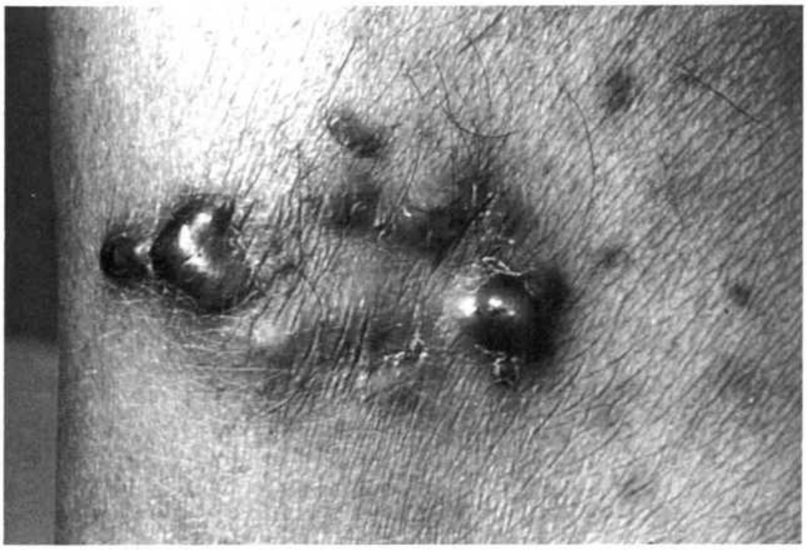

FIGURE 1. Purplish nodules on the skin.

Histology. There were many dilated blood vessels with a proliferation of spindle cells in the dermis.

Treatment. The immunosuppressive therapy was reduced, but the lesions of Kaposi's sarcoma did not improve. After the immunosuppressive therapy was discontinued the renal function deteriorated and the patient had the transplant removed. Even after discontinuation of the immunosuppressive therapy the tumours continued to grow and he had electron-beam therapy with a total dose of 35 Gy. A complete remission soon occurred which has continued.

Discussion. Renal transplant recipients are more at risk from the development of certain forms of cancer. The incidence of Kaposi's sarcoma is 50–500 times higher when compared with the normal population.[1,2] Cytomegalovirus infection is believed to play an important role in the development of Kaposi's sarcoma. The treatment of choice for Kaposi's sarcoma in renal transplant recipients is a reduction or stopping of the immunosuppressive therapy.[3] Irradiation, surgery and chemotherapy have been used, with varying degrees of success. This patient responded very well on subtotal skin irradiation with electron beams.

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Traumatic gunpowder tattoo

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History. A 11-year-old boy developed traumatic tattoos on the face from the accidental discharge of a starting pistol.

Examination. Multiple grey–black pigmented macules varying in size from 1 to 2 mm in diameter in the left orbital region developed 3 months after the accident.

Comments. Traumatic tattoos occur with the deposition of pigmented particles in the dermis.[1] Carbon is the most common material deposited and, depending on the depth in the dermis, gives a black or blue colour. The foreign material may be embedded after an explosion or following abrasive trauma such as after road accidents. In the acute stage, saline compresses are recommended for removing the fragments but after 7 days, when the skin is less indurated, dermabrasion can be performed. This should be restricted to the papillary dermis to ensure rapid regeneration of the epidermis and to minimize scarring.[2] Alternative therapies such as laser treatment and abrasion with sandpaper followed by the application of tannic acid and silver nitrate have been used.

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Granulomatous slack skin

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History: A 58-year-old male over the past 9 years had slowly developed bulky erythematous skin folds on the abdomen and left chest area and axillae. These were diagnosed and treated elsewhere as eosinophilic granuloma.

Examination. On the left part of the chest and on the left upper arm and in the groins there were poikilodermatous, atrophic and erythematous bag-like drooping folds of skin. On the back and in the lumbosacral area there were erythematous, discrete scaling plaques. On the right hip there was an ulcerated plaque that was very similar to infection by mycosis fungoides. In the groins there were several small lymph nodes.

Laboratory. Routine investigations showed an ESR of 30 mm and there was 10% blood eosinophilia. The immunoglobulins were normal. Lymphocyte transformation tests with phytohaemoglobulin, poke weed mitogen and ConA showed decreased values and stimulation with *Candida albicans*, PPD, tetanus toxoid and varidase showed negative results.

Histology. In the dermis there was a dense infiltrate of lymphocytes, histiocytes and locally, numerous eosinophils. Throughout the dermis there were granulomas that contained multinucleated giant cells. An elastic stain showed extensive destruction of the elastic fibres and remnants were present in the giant cells. Nuclear dust was present around some of the dermal vessels.

Treatment. The patient was treated with prednisone 15 mg daily and on reducing the dose, his condition deteriorated and he became febrile. Later he had an adenocarcinoma of the colon removed and during this operation a very large spleen (weight 800 g) was excised. The spleen showed many granulomas which were also found in the skin. Subsequently the patient developed various facial skin malignancies that included: one fibrosarcoma, four basal cell carcinomas and four squamous cells carcinomas, all of which were treated appropriately. The patient developed a recurrence of a squamous cell carcinoma and a lymph node was excised. There was no evidence of malignancy in this lymph node, but numerous granulomas were found. After 5 years the patient died unexpectedly. Autopsy was not permitted.

Discussion. Granulomatous slack skin is characterized by the slow evolution of bulky skin lesions which ultimately result in lax pendulous folds of skin. Histologically it is characterized by a dense granulomatous infiltrate and by destruction of the elastic fibres.[1] Nine patients have so far been reported, three of them with a Hodgkin's lymphoma and one a lymphoblastic non-Hodgkin's lymphoma. Granulomatous lesions resembling those seen in the skin, in other tissues like the spleen and lymph node as in our patient have not been reported before. Granulomatous slack skin probably represents a slowly progressive lymphoproliferative disorder.[2]

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Brachytherapy for satellite metastasis of a malignant melanoma of the penis

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History. A 68-year-old man had had for one year a verrucous lesion on the penis. In the previous 3 months it had rapidly increased in size and had bled. The patient had worked as an actor in sado-masochistic shows.

Examination. On the glans penis there was a 1 cm diameter red-glossy tumour which bled. (Fig. 1). No lymph nodes were palpable.

Histology. A biopsy from the tumour on the penis showed the histology of a malignant melanoma.

Therapy and course. Because the patient refused a partial or total penectomy the tumour was removed by a large total excision. A year later three small brown macules were present on the glans penis. The histology of these new lesions was similar to that of the biopsy of the primary lesion. No extracutaneous lesions were present and the patient again refused amputation of the penis. The satellite metastases of the malignant melanoma of the penis were treated with brachytherapy using iridium 192 wires (Fig. 2). Later lung and liver metastases were demonstrated but so far there has been no recurrence of the melanoma on the penis.

Discussion. Primary malignant melanoma of the penis is rare. The treatment is surgical and a total amputation of the penis with removal of the inguinal lymph nodes has been advocated.[1] Since 1961 brachytherapy with iridium 192 wires[2] has been used to treat squamous cell carcinoma of the penis of 4 cm or less. To our knowledge this is the first report of a patient with a penile melanoma, treated by brachytherapy.

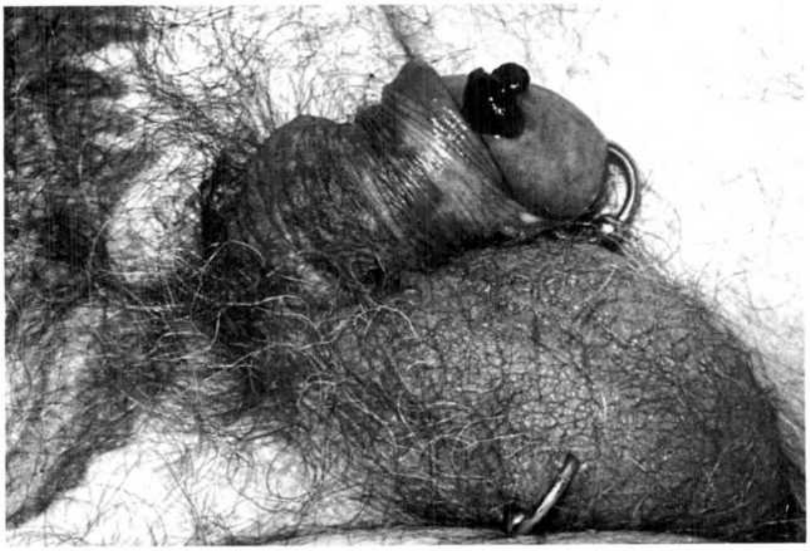

FIGURE 1. A bleeding malignant melanoma of the glans penis.

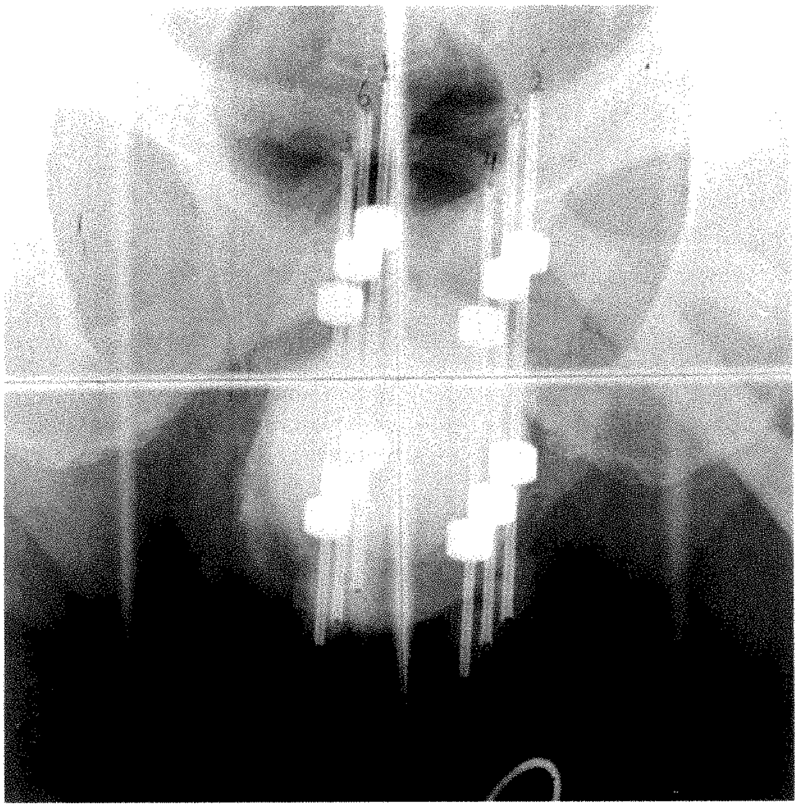

FIGURE 2. AP X-rays of the interstitial inplants of the needles used as vectors for the iridium wire.

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Inherited deficiency of the third component of complement, associated with cutaneous lupus erythematosus

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History. A female patient aged 27 had, during infancy, suffered from recurrent otitis media and at 21 months had pneumococcal meningitis. From the age of 6 she was on long-term ampicillin and remained well up until the age of 17 when the ampicillin was discontinued and she had a recurrence of her otitis media. She also had a transient maculopapular rash during the episodes of infection. After the diagnosis of congenital C3 deficiency was made, antibiotic prophylaxis was resumed.

A total C3 deficiency was also found in her two sisters, who also had recurrent episodes of bacterial infections. The other family members, including both parents, had (sub)normal levels of C3. One brother had died from bacterial meningitis at the age of 3.

In September 1986 the patient developed skin lesions on the face and upper trunk.

Examination. There was a butterfly rash on the cheeks and nose (Fig. 1). On the upper chest (Fig. 2) and back there were discoid erythematous macules and plaques.

Histology. The epidermis was flat with few rete pegs and there was follicular plugging. The basal cell layer showed vacuolar degeneration and in the dermis there was a perivascular and perifollicular lymphocytic infiltrate.

Immunofluorescence showed band-like deposits of IgM and membrane attack complex of complement (MAC), but no C3.

Investigations. ESR 5 mm/h, platelets 131×10^9/l, positive anti-nuclear factor. CH50 4 U/ml, Clq 15 mg%, C2 390 U/ml, C3 0 mg%, C4 28 mg%. Clq-binding assay 27 μgeq/ml, IgA-polyethylene glycol assay 239 μgeq/ml.

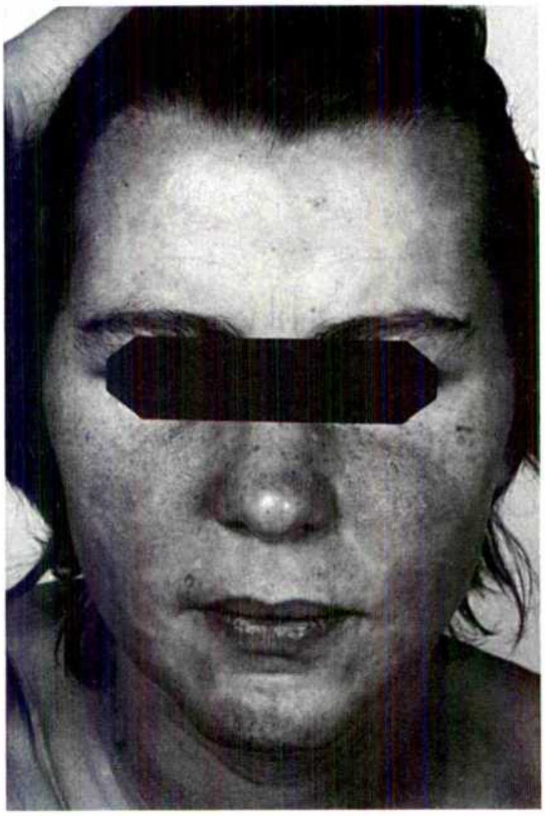

FIGURE 1. Butterfly rash on the face.

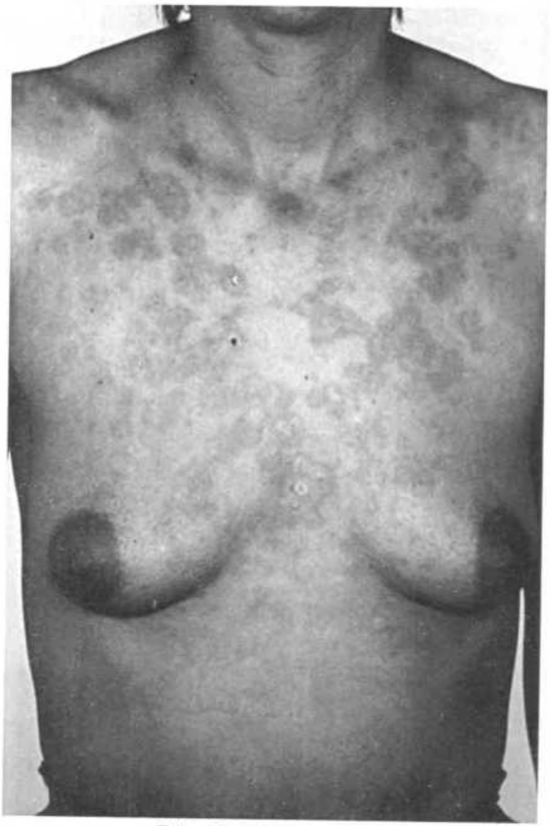

FIGURE 2. Discoid lesions on the upper chest.

Discussion. C3 deficiency is inherited as an autosomal recessive gene with the heterozygotes having subnormal levels of C3[1] but without any health problems. Apart from recurrent bacterial infections homozygote C3 deficiency is characterized by bouts of fever, exanthema rashes, arthralgia, and vasculitis. These symptoms, which are probably caused by the presence of insoluble immune complexes respond to treatment with whole blood.[2] Occasionally, proteinuria and hematuria can be present.

The associations between complement deficiency, with immune complex disorders and lupus erythematosus (LE) have long been recognized.[3] It seems to be a paradox that absence of a properly functioning complement system predisposes for LE, since complement plays an important role in its pathogenesis.

In order to explain the high incidence of LE among patients with complement deficiencies, it has been suggested that the close linkage of genes which encode for certain complement components (C2, C4) and the HLA-locus on the short arm of chromosome 6 is a factor.[4] For patients with a congenital C3 deficiency this cannot be true, since the C3 gene is located on chromosome 19.

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Pili torti as a marker of Rapp-Hodgkin ectodermal dysplasia syndrome in three generations

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History. Male, aged 7 years. The boy was born after a normal full-term pregnancy. At the age of one year a bilateral subtotal cleft palate deformity was closed. Recurrent otitis media made pressure equalizing tubes necessary. There was no history of heat intolerance. His hair and nails grew slowly and his dental development was delayed.

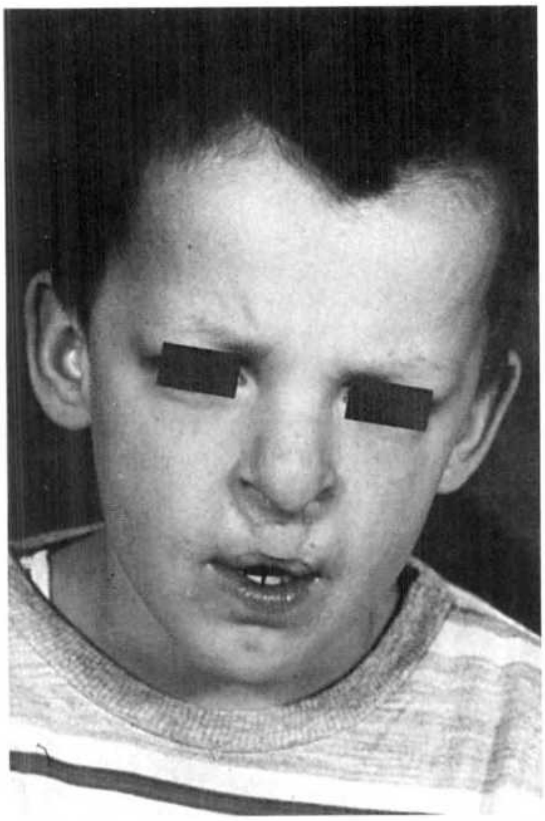

FIGURE 1. Rapp–Hodgkin ectodermal dysplasia.

Examination. The patient had the typical facial features with high forehead, narrow nose, small mouth and maxillary hypoplasia (Fig.1). The hair was coarse and wiry. The lateral eyebrows were sparse. The teeth were small and the finger- and toe-nails were dysplastic. There was a glanduar hypospadia. The hair shafts showed irregular twists around the long axis such as seen in pili torti.

Discussion. Examination of the proband's mother, grandmother and one of the mother's sisters revealed the same typical oro-facial features, sparse hair of the lateral eyebrows and dystrophic nails. There was no cleft palate. The teeth of mother and grandmother were lost at an early age and those of the aunt were dystrophic and carious. The mother had coarse scalp hair with unusual light reflections. The axillary and pubic hair were sparse. The grandmother had an extensive alopecia of the scalp. An aunt had normal hair but light microscopy of the hair shafts from all three showed pili torti.

Two other sisters of the proband's mother and the son of one of them were also examined. They had no symptoms or signs of ectodermal oro-facial dysplasia and the hair shafts showed no light microscopic abnormalities.

Rapp–Hodgkin ectodermal dysplasia syndrome is thought to be inherited in an autosomal dominant fashion.[1] Light microscopy of the hair shafts made it possible to make a distinction between affected and non-affected members of the family.[2]

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SUMMARIES OF PAPERS

Mutational activation of *ras* oncogenes in basal cell carcinomas and squamous cell carcinomas

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Activation of oncogenes can induce malignant transformation, which may lead to the development of carcinomas and other malignant tumours. Activated *ras* oncogenes have been identified in a variety of malignant tumours. Activation of *H-*, *K-*, and *N-ras* genes has been shown to occur almost exclusively by point mutations in the codons for amino acids 12, 13 and 61.

In our study we investigated whether such mutations can be detected in DNA obtained from basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) of human skin. DNA was isolated from incisional biopsies from 17 BCC and 5 SCC. By using the polymerase chain reaction, the *ras* gene-containing sequences of genomic DNA were amplified. The DNA samples were subsequently spotted on to nylon filters and hybridized to a panel of ^{32}P-labelled synthetic oligonucleotides, which are complementary to either the non-mutated sequences of the *ras* genes or to each of the possible point mutations.

The results showed that point mutations were found in *K-ras* and *H-ras* genes of three BCC and one SCC. In two BCC and in one SCC the mutations were found in codon 12 of the *K-ras* gene. In another BCC a mutation was found in codon 61 of the *H-ras* gene. These mutations in the *K-ras* gene result in a replacement of glycine by cysteine at position 12 of the gene product. The mutation in the *H-ras* gene resulted in a replacement of glutamine by histidine at position 61 of the geneproduct.

These results demonstrate that mutational activation of *ras* oncogenes can occur in carcinomas of the skin. This finding may explain the development of malignant degeneration in at least a part of skin carcinomas.

Fumaric acid therapy for psoriasis

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Fumaric acid therapy for psoriasis was first proposed in 1959 by the German chemist Schweckendiek[1] and further developed by the German physician Schäfer.[2] The therapy consists of oral treatment with monoethyl- and dimethylfumarate and topical monoethylfumarate. In addition, a diet is prescribed. There has been much interest in therapy with fumaric acid by Dutch patients with psoriasis.[3]

We conducted a double-blind, placebo-controlled study to investigate efficacy and side-effects of this therapy. Thirty-nine patients were randomly allocated to oral treatment for 16 weeks with either a combination of dimethylfumarate and different salts of monoethylfumarate (Fumaderm forte®), with octyl hydrogen fumarate, or with placebo. Five patients dropped out from the study because of side-effects or worsening of their skin lesions. The percentage of body surface affected with psoriasis and the scores for infiltration and scaling were markedly reduced in those on treatment with Fumaderm forte®. Seventy-five per cent of the patients treated with Fumaderm tablets had a good result. This was significantly better than obtained with placebo or with octylfumarate.

The main side-effects were flushing and diarrhoea. Laboratory investigations revealed disturbances of the liver function, lymphocytopenia and eosinophilia and one patient developed disturbance of the renal function that was reversed on stopping treatment.

A second study was designed to investigate the clinical efficacy and side-effects of the separate components of Fumaderm forte® in comparison with Fumaderm forte®. This study was terminated after a few weeks because of severe renal side-effects. All the side-effects observed in the first study were also encountered in this study, although diarrhoea was seen only in the patients treated with dimethylfumarate. The renal problems were more serious in the patients treated with monoethylfumarate. A rise in serum transaminases was seen only in patients treated with dimethylfumarate.

At present 68 patients suffering from psoriasis are being treated with Fumaderm® tablets. Seventy per cent of these patients have responded well to treatment. With long-term treatment the gastrointestinal symptoms disappear but the flushing persists. Ten of the patients on maintenance therapy complained of tiredness.

Fumaric acid therapy is an effective treatment for psoriasis that in most patients is well tolerated. Disturbances of renal function occur which are reversible. Further studies are needed to determine long-term toxicity.

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Intracutaneous tests with *Pityrosporon* extract in atopic dermatitis

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Yeasts of the genus *Pityrosporon* (*Malessezia furfur*) are known to constitute a major proportion of the saprophytic microflora of human skin and are associated with a number of disease conditions including pityriasis versicolor, folliculitis, seborrhoeic dermatitis, dandruff, acne vulgaris and systemic infections in immuno-compromised patients. Because there are indications that *Pityrosporon orbiculare* is of importance in cases of atopic dermatitis, we performed prick- and intracutaneous tests with *Pityrosporon* extract in 57 patients suffering from this condition.[1,2] The same test was carried out on 52 controls that consisted of 41 atoptic patients with asthma and/or rhinitis, seven non-atopic patients with allergic contact dermatitis and four non-atopic patients with seborrhoeic dermatitis or pityriasis versicolor. Specific IgE against *Pityrosporon* was determined in these patients by means of RAST.

There were statistically significant differences in the results obtained in the two groups. In the patients with atopic dermatitis the intracutaneous test was 87·5% positive; the prick test 59% positive and the RAST 65% positive to *Pityrosporon* extract. In the atopic controls these numbers were 12, 0 and 0% respectively. All the non-atopic control patients showed negative reactions, both in the skin tests and in the RAST. Skin tests with *Pityrosporon* extract and specific IgE determinations may contribute to the diagnosis of atopic dermatitis and *Pityrosporon* may be of significance in the pathogenesis of this disease.

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Furunculosis and hypoferraemia

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Furunculosis caused by *Staphylococcus aureus* may occur as a sign of underlying disease. The relationship of low serum-iron and this chronic staphylococcal infection was studied in three groups of patients: 16 patients with several furuncles, eight with one single furuncle and 10 patients with longstanding and severe acne conglobata.

The serum-iron level was significantly lower in those with multiple furuncles as compared with the others where it was normal. All laboratory investigations including the haemoglobin were normal. Treatment with oral iron preparations resulted in an improvement in all the patients.

British Journal of Dermatology (1989) **121**, 817–822.

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Palmar and plantar pitting in psoriasis

SIR, We describe a man with psoriasis treated with etretinate who developed palmar and plantar pits.

A 70-year-old man first presented in 1975 with eczematous dermatitis of the face, neck and dorsa of the hands. This was thought to be contact dermatitis that was associated with his work as a grinder. The skin rash cleared in a month with topical steroid preparations and he was not seen again until 1985 when he presented with gross hyperkeratosis and fissuring of the palms and soles, scaling of the scalp, a mixed eczematous/psoriasiform eruption on the trunk and limbs and a few nail pits that were characteristic of psoriasis. He was admitted to hospital and successfully treated with topical steroid to the palms and soles and dithranol in Lassar's paste to the trunk and limbs. His psoriasis was reasonably controlled with various topical preparations until he developed an acute exacerbation of widespread psoriasis in September 1987, soon after he retired from work. He was started on oral etretinate in November, initially 1 mg/kg per day for 2 months, 0·6 mg/kg per day for 3 months and subsequently 0·5 mg/kg per day. White soft paraffin containing 20% metosyn was used on the palms and soles. After 7 months on treatment with etretinate his psoriasis was almost clear but he was noted to have asymptomatic, regularly spaced small pits on both palms (Fig 1). The soles were similarly but less markedly affected. He also, at this time, showed increased

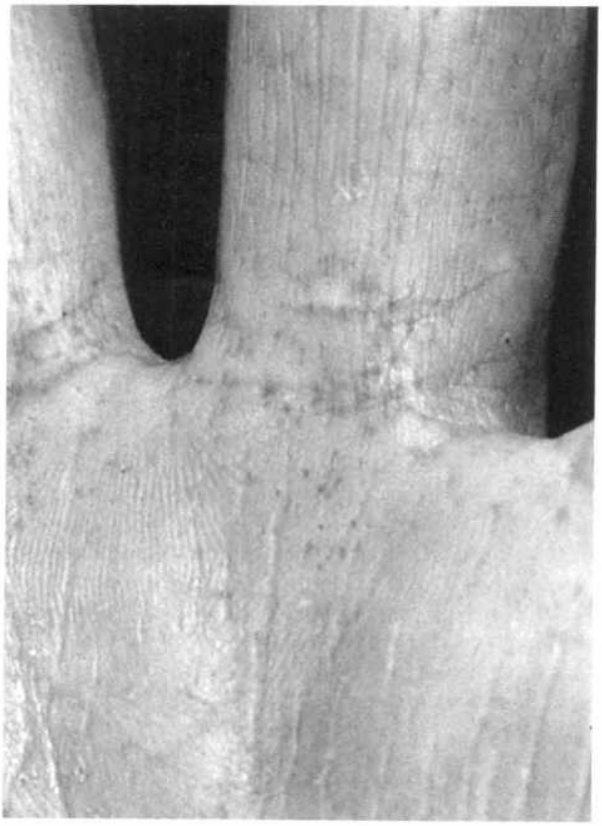

FIGURE 1. Palmar pitting.

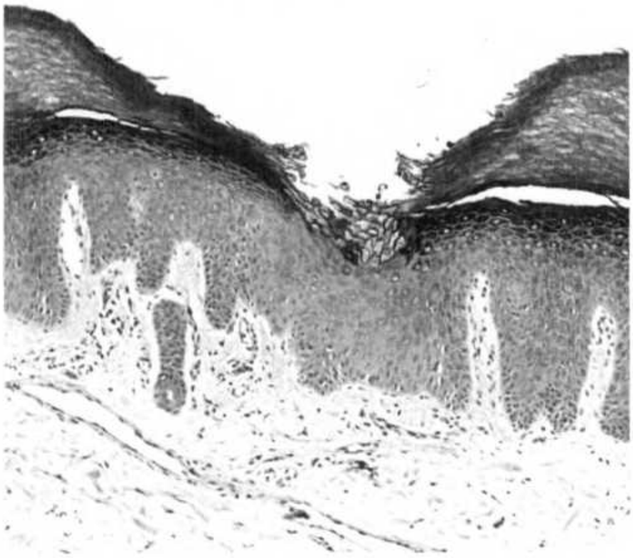

FIGURE 2. Histology of a palmar pit.

pitting of his nails. Radiographs of his mandible and maxilla did not show dental cysts nor were there bifid ribs. His daughter has moderately severe eczema but there was no other family history of skin disease.

Histology of the palmar pits (Fig. 2) showed that the keratin layer was almost completely lost at regular intervals leaving V-shaped depressions. At the base of the pits the cells in the stratum corneum were mildly enlarged and the granular layer was absent at these points. There was no consistent relationship of the pits to eccrine sweat glands. Mildly dilated capillaries were present in the dermal papillae with a sparse chronic inflammatory cell infiltrate.

To our knowledge palmar and plantar pits have not been reported previously in psoriasis. Involvement of the finger nail in psoriasis is common and has been reported in up to 50% of cases.[1] Nail plate abnormalities include 'pitting' and our patient has psoriasis with typical nail pitting. However, the aetiology of his palmar and plantar pits is unknown. They could be related to psoriasis and/or the etretinate treatment or coincidental. The main differential diagnoses are the basal cell naevus syndrome and Darier's disease, but the pits in these disorders can be differentiated clinically from those in our patient by their elevated margins.[2] They are also histologically distinct.[3-5] The palmar and plantar lesions seen in pitted keratolysis, keratosis punctata of the palmar creases, keratosis palmaris et plantaris and arsenical keratoses are also distinct by being palpable, as are the papular lesions seen on the palms and soles in porokeratosis of Mibelli and porokeratosis of Mantoux.

Our patient had been on oral etretinate for 7 months when the palmar and plantar pits were first noticed. Although his rash had generally cleared, nail pits were also present. Increased skin fragility with fragmented, loose stratum corneum is a frequent side-effect of the retinoids, isotretinoin and etretinate,[6] but there have been no reports of localized lesions such as pits occurring in patients on retinoids.

There is evidence in favour of an association with his psoriasis in the similarity of the histology of the palmar pitted skin with that seen in the nail matrix. Lewin *et al.*[7] using the method described by Zaias[8] performed a detailed study of the pathology of the finger-nail matrix in psoriasis. The resulting abnormality depends on the portion of the nail affected and the extent to which it is involved. Nail pits result when only the proximal part, which produces the superficial layers of the nail plate, is involved. Parakeratotic cells, which overlie a lost granular layer are incorporated into the nail plate. They are poorly adherent to each other and are easily dislodged by minor trauma, thus forming the nail pits. This is very similar to the histology in our case where, although no parakeratotic cells were seen, there was no granular layer and the parakeratotic cells could be presumed to have been dislodged leaving the pits or defects in the keratin layer. Our patient's palmar and plantar pits may represent previously unreported psoriatic lesions similar in formation to psoriatic nail pitting.

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Post-irradiation morphoea

SIR, I should like to comment on the interesting paper by Colver *et al.*[1] in which they describe nine female patients, seven with carcinoma of the breast, who developed morphoea after radiotherapy.

Recently we described four patients in whom scleroderma (systemic sclerosis in three and morphoea in one) developed within 18 months of detection of breast carcinoma. Two patients, one with systemic sclerosis and one with morphoea, had received radiotherapy.[2]

An earlier report[3] described systemic sclerosis appearing within 6 months of radiotherapy-treated carcinoma in two patients and two patients in whom systemic sclerosis preceded the breast carcinoma by less than 2 years. Thus, although an association between development of morphoea and prior radiotherapy appears real there is also a wider relationship between carcinoma and scleroderma.[4,5]

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Nicardipine interacts with cyclosporin

SIR, The recent report by Griffiths *et al.*[1] on the long term use of cyclosporin in psoriasis draws attention to the frequent development of hypertension during therapy. This often requires treatment with hypotensive agents such as calcium-channel blockers, including nicardipine. We report the case of a patient on long-term cyclosporin for dermatomyositis whose blood cyclosporin levels rose dramatically following the introduction of nicardipine. An interaction between cyclosporin and nicardipine has been noted in a few renal transplant recipients.[2]

The 30-year-old patient was maintained in remission on cyclosporin 200 mg per day. The trough blood levels of cyclosporin (Sandoz radio-immunoassay on whole blood using specific and non-specific antibodies) remained stable on this dose for 11 weeks. On becoming hypertensive she was initially treated with atenolol 100 mg per day for 10 days and then metoprolol 200 mg per day. Since this produced only a partial response nicardipine 100 mg t.d.s. was added to her therapy. Within 16 days the cyclosporin levels had risen by almost 100% and remained elevated over the next 4 weeks until the dose of cyclosporin was reduced (Fig. 1).

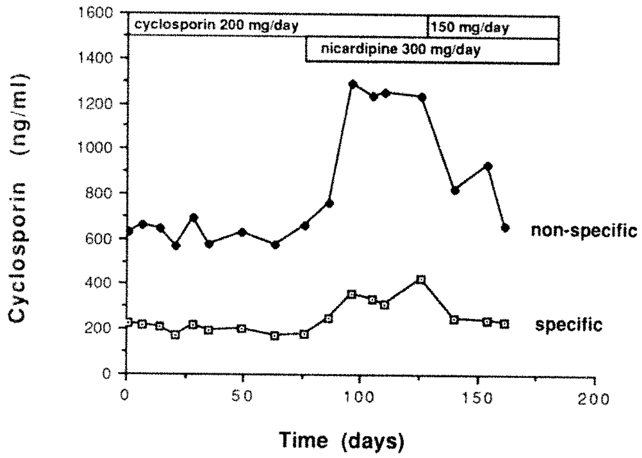

FIGURE 1. Trough blood levels of cyclosporin during treatment with cyclosporin and cyclosporin plus nicardipine.

It seems likely that cyclosporin will be used with greater frequency for skin conditions that have failed to respond to conventional treatment. Hypertension will be increasingly encountered as an unwanted side-effect. Awareness of the interaction described here is important in order that cyclosporin levels may be safely monitored when nicardipine is used as a hypotensive agent.

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Pityriasis rubra pilaris in Indians

SIR, The clinical pattern of pityriasis rubra pilaris in white races is well recognized,[1-3] but only a few cases have been reported in those with racially pigmented skin. The disease may occur as exfoliative dermatitis[4] with islands of normal skin; this being uncommon in Indians, with only six cases reported among 350,000 out-patients seen in the past decade.(Table 1). The patients had mild to moderate itching, and the rash started mainly with scaling on the scalp and in three there was palmar and plantar keratoderma. The age of onset was from 11 to 28 years and the duration of the disease was variable. There was extensive erythema with mild to moderate scaling and follicular papules (Fig. 1) that were associated with a palmoplantar keratoderma. In three of the patients there was an exfoliative dermatitis. The nails were thickened and

TABLE 1. Clinical characteristics of pityriasis rubra pilaris

Age at onset (years)	Duration of disease (years)	Skin lesions	Nails	Scalp
22	6	Discrete and confluent scaly erythema with follicular papules and palmoplantar keratoderma	Thick brittle nails with subungual hyperkeratosis	Mild scaling
25	0·1	Discrete follicular papules with fine white scales and erythema. Dryness and cracking of skin and palmoplantar keratoderma	Unaffected	Unaffected
17	0·25	Discrete and confluent follicular papules with scaly erythema on dorsum of hands, elbows, knees	Thickened nail plates with frayed distal edge and mild subungual hyperkeratosis	Diffuse scaling with erythema and patchy hair loss
18	7	Generalized fine scaling with follicular plugging sparing the periorbital nasal and malar areas. Palmoplantar hyperkeratosis	Thickened nail plates with mild subungual hyperkeratosis	Mild scaling
28	8	Discrete/confluent acuminate follicular papules, diffuse scaling with hyperpigmentation. Palmoplantar keratoderma	Thickened nail plates, with ridging pitting and subungual hyperkeratosis	Generalised mild scaling with hair loss
11	3	Generalized greyish white scaling with underlying erythema and oedema. Palmoplantar keratoderma	Thickened nail plates with longitudinal ridging	Hair loss especially of eyebrows and eyelashes

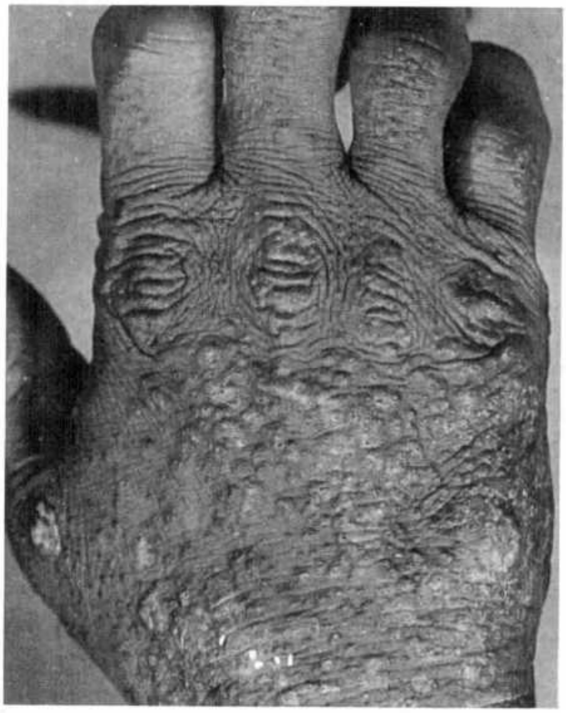

FIGURE. 1. Acuminate follicular papules over dorsum of the hand.

brittle with subungual hyperkeratosis. Though the condition of pityriasis rubra pilaris is rare, we wish to report these cases in Indians.

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Book Review

Pharmacology of the Skin, Vol. 1. Edited by M.W.GREAVES AND S.SHUSTER (1989). Berlin: Springer-Verlag. 78 figures. Pp. 495. ISBN 354019403/7. Price 480 DM.

This is the first volume of an ambitious and comprehensive two-volume work on the pharmacology of the skin. Volume 1 consists of 29 chapters covering the pharmacology of skin systems and their control and also the role of autocoids in normal and inflamed skin. The second volume will include chapters on the methods of measurement in studies on skin and skin disease as well as toxicology and descriptions of individual drugs and drug groups. The editors hope that the volume will appeal to a wide range from pharmacologists and research scientists to dermatologists and industrial researchers. I think they are optimistic in believing that honours undergraduate students and Ph. D. students will make great use of it. There are some very authoritative well written and up-to-date contributions, e.g. the chapter on mast cells. Other topics are dealt with in a more superficial manner and are unlikely to achieve the Editor's aim of an up-to-date review giving sufficient background without having to refer beyond the two volumes. The book, however, does bring together a lot of information about drug research and the skin and should prove valuable to both basic and clinical researchers.

J.REID

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News and Notices

British Dermatological Surgery Group VIIth Annual Practical Workshop
29–30 March 1990, Belfast

The British Dermatological Surgery Group will be holding its seventh Annual Practical Workshop in Belfast. This is a course in basic dermatosurgery with lectures and supervised practical experience. It is open to all dermatologists, consultants, trainees and clinical assistants. For further information contact Dr Ann Bingham, Department of Dermatology, Royal Victoria Hospital, Grosvenor Road, Belfast BT12 6BA.

Enquiry into Children as Day Case Admissions

The Nuffield Foundation is funding an enquiry into children as day case admissions, to be carried out by the Caring for Children in the Health Services Committee. Surgical, medical and psychiatric admissions will be included and the aim is to identify a comprehensive set of multidisciplinary standards which can be used to assess the quality of care provided before admission, within the hospital and following discharge. The Committee will be accepting submissions from organizations and individuals. Information from Mrs R. Thornes, 7 Belgrave Place, Clifton, Bristol BS8 3DD.

70th Annual Meeting of the British Association of Dermatologists:
18–21 July 1990

The next Annual Meeting of the British Association of Dermatologists will be held at the University of Warwick, Coventry, on 18–21 July 1990 under the presidency of Dr R.R.M.Harman.

Abstracts are invited for consideration by the Selection Committee. These must be submitted on official abstract forms which are available from Dr M.M.Black, Honorary Secretary, British Association of Dermatologists, 3 St Andrews' Place, London NW1 4LB. The closing date for receipt of completed abstract forms is Friday 19 January 1990.

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